PROCEEDINGS OF VI. INTERNATIONAL AGRICULTURAL, BIOLOGICAL, LIFE SCIENCE CONFERENCE AGBIOL 2024

18-20 SEPTEMBER 2024

EDIRNE, TURKEY

PROCEEDINGS OF VI. INTERNATIONAL AGRICULTURAL, BIOLOGICAL, LIFE SCIENCE CONFERENCE AGBIOL 2024

18-20 SEPTEMBER 2024
EDIRNE, TURKEY

Organized by
Trakya University
International Researchers Association

ISBN #:
978-625-95132-1-8

WELCOME NOTES

You are welcome to our VI. AGBIOL Conference that is organized by Trakya University. The aim of our conference is to present scientific subjects of a broad interest to the scientific community, by providing an opportunity to present their work as oral or poster presentations that can be of great value for global science arena. Our goal was to bring three communities, namely science, research and private investment together in a friendly environment of Edirne, Turkey in order to share their interests and ideas and to get benefit from the interaction with each other.

In September 2018, we organized the first AGBIOL Conference with more than 700 scientists and researchers from all over the world with over 800 scientific papers. Due to COVID-19 situation, II. AGBIOL 2020 has organized fully on-line event which was one of the biggest online conferences in recent years in the world with 499 papers and 1133 authors with 333 oral and 166 e-poster presentations from 55 countries. Due to COVID-19 situation, AGBIOL 2021 was organized online again. AGBIOL 2022 conference was organized with a worldwide participation from 44 countries over 522 papers contributed by over 1300 authors. AGBIOL 2023 was organized with a record and worldwide participation from 33 countries 833 papers contributed by over 2000 authors with 522 oral and 311 poster presentations.

There is a worldwide participation from 55 countries 835 papers contributed by about 2000 authors with 522 oral and 311 poster presentations in AGBIOL 2024.

The AGBIOL 2024 is normal participation as well as with online participation in Trakya University Balkan Congress Center in Edirne, Turkey on 18-20 September, 2024. The program included oral talks by invited prominent scientists and oral and e poster presentations by participants in selected topics from the submitted abstracts focusing on Agriculture, Biology and Life Sciences topics.

With care for our nature and environment, we aim the green congress, meaning that as little as possible papers will be used. Abstract book is published in electronic book and is distributed to the participants by e mail for online participants. All the e-posters are prepared in electronic form and then submit to via the conference e mail and exhibited in electronical poster boards as well as in online e poster hall in our web page during the conference.

The participants with paid conference fee accesssed all the normal and virtual presentation talks in each session, as well as to visit the virtual poster hall via preliminary provided. The abstracts were published in the Conference Abstract and Proceedings Book. Participants might send us their full papers, which based on their preferences are published either in our Conference Abstract and Proceedings Book or in selected International Indexed Scientific Journals.

Conference Topics:

Agriculture, Forestry, Life Sciences, Agricultural Engineering, Aquaculture and Biosystems, Animal Science, Biomedical science, Biochemistry and Molecular Biology, Biology, Bioengineering, Biomaterials, Biomechanics, Biophysics, Bioscience, Biotechnology, Botany, Chemistry, Chemical Engineering, Earth Sciences, Environmental Science, Food Science, Genetics and Human Genetics, Medical Science, Machinery, Pharmaceutical Sciences, Physics, Soil Science.

We would like to thank all of you for joining this conference and we would like to give also special thanks to our sponsors and collaborators for giving us a big support to organize this event.

Prof Dr Yalcin KAYA
Head of the Organizing Committee

ORGANIZING COMMITTEE

Prof. Dr. Yalcin KAYA	Trakya University, Turkey	Chair
Assoc Prof. Dr. Necmi BEŞER	Tragen Co, Trakya Technopark, Turkey	Co-Chair
Emrah AKPINAR	Trakya University, Turkey	Secretary
Prof. Dr. Ahmet ULUDAG	Onsekizmart University, Turkey	Member
Prof. Dr. Metin TUNA	Namık Kemal University, Turkey	Member
Prof. Dr. Viliana VASSILEVA	Inst. of Forage Crops, Pleven, Bulgaria	Member
Prof. Dr. Ioannis TOKATLIDIS	Trakia Democritus University, Greece	Member
Prof Dr Mohamed RAMDANI	University of Mohamed V Agdal, Maroc	Member
Assoc. Prof. Dr. Natiga NABIYEVA	Genetic Resources Inst. of NAS, Azerbaijan	Member
Asst Prof Dr Orhan ASKIN	Kırklareli University, Turkey	Member
Dr Maria PACUREANU	NARDI Fundulea Institute, Romania	Member
M. İbrahim YILMAZ	Trakya Agricultural Research Inst., Turkey	Member

INVITED SPEAKERS

Prof Dr Fikrettin Şahin, Yeditepe University, Istanbul, Turkey

Prof Dr Nikolay D. Panayotov, Agricultural University of Plovdiv, Bulgaria

Prof Dr Ahmet Uludağ, Çanakkale Onsekizmart University, Turkey

Prof Dr Mehmet Emin Çalışkan, Niğde Omer Halisdemir University, Turkey

Assoc Prof Dr Amel NAJJAR, University of Carthage, National Institute of Agronomy, Tunisia

Asst Prof Dr Sarra JRIBI, University of Carthage, National Institute of Agronomy, Tunisia

EDITOR OF THE PROCEEDINGS ABSTRACT BOOK

Prof Dr Yalcin KAYA, Assoc Prof Dr Necmi BESER

SCIENTIFIC COMMITTEE

NAME	INSTITUTION	COUNTRY
Acad. Prof. Dr. Atanas ATANASSOV	Joint Genomic Center - Sofia	Bulgaria
Prof Dr Amel MIlLA	ENSV, Alger	Algeria
Prof. Dr. Miguel CANTAMUTTO	IINTA Hilario Ascasubi Inst.,	Argentina
Prof. Dr Zhao JUN	Inner Mongolia Agric. Un.	China
Prof. Dr. Renata HORN	University of Rostock	Germany
Prof. Dr. Mulpuri SUJATHA	Indian Inst of Oilseeds Res.	India
Prof. Dr Lara HANNA WAKIM	Holy Spirit University	Lebanon
Prof. Dr Semra HASACEBI	Trakya University	Turkey
Prof Dr Velibor SPALEVIC	University of Montenegro	Montenegro
Prof Dr Charles BRENNAN	RMIT University	Australia
Prof. Dr. Saeed RAUF	University of Sargodha	Pakistan
Prof. Dr. h.c. Radu E. SESTRAS	Agric.Vet Un Med Cluj-Napoca	Romania
Prof. Dr. Dejana PANKOVIC	Educon University	Serbia
Prof Dr Leonardo VELASCO	Inst. for Sustainable Agric. CSIC	Spain
Prof. Dr. Mehmet Emin CALISKAN	Nigde OmerHalisdemir Univ.	Turkey
Prof. Dr Ahu ALTINKUT UNCUOGLU	Marmara University	Turkey
Prof. Dr. Sezen ARAT	Namık Kemal University	Turkey
Prof. Dr. Coskun GULSER	Ondokuzmayis University	Turkey
Prof. Dr. Metin AYDOĞDU	Trakya University	Turkey
Prof. Dr. Ismail CAKMAK	Sabanci University	Turkey
Prof. Dr. Yaroslav BLUME	National Academy of Sciences	Ukraine
Prof. Dr. Nurhan T. DUNFORD	Oklahoma State University	USA
Prof. Dr. Mahmut TOR	University of Worcester	England
Prof. Dr Ozlem TOKUSOGLU	Celal Bayar University	Turkey
Prof. Dr Mustafa TAN	Trakya University	Turkey
Prof. Dr. Gökhan KAÇAR	Trakya University	Turkey
Prof. Dr. Süleyman KÖK	Trakya University	Turkey
Assoc. Prof. Dr Zizis VRYZAS	Democritus Univ. of Thrace	Greece
Assoc. Prof. Dr. Nooduan MUANGSAN	Suranaree Univ. of Technology	Thailand
Asst. Prof. Dr. Sarra JRIBI	University of Carthage	Tunusia
Asst. Prof. Dr. Buket AŞKIN	Kırklareli University	Turkey
Asst. Prof. Dr. Hayati ARDA	Trakya University	Turkey
Asst. Prof. Dr. Güzin TUNCA ALPASLAN	Trakya University	Turkey
Asst. Prof. Dr. Nihan Bilge KAMER	Trakya University	Turkey
Asst. Prof. Dr. Mehmet YABAŞ	Malatya Turgut Özal Univ.	Turkey

CONTENTS

EFFECTS OF EDIBLE MUSHROOM Hohenbuehelia petaloides (Bull.) Schulzer on APOPTOSIS AND CELL CYCLE IN HT-29 CANCER CELL LINE

Cansu KORKMAZ[1*], *Mehmet Emin DURU*[2], *Hatice GÜNEŞ*[1]

[1]*Department of Biology, Faculty of Science, Muğla Sıtkı Koçman University, 48000 Muğla, Türkiye,*
[2]*Department of Chemistry, Faculty of Science, Muğla Sıtkı Koçman University, 48000 Muğla, Türkiye,*

Corresponding author e-mail: cansukorkmaz08@gmail.com
https://orcid.org/0000-0003-3027-7687

ABSTRACT

In addition to being low in calories, mushrooms are important natural sources of essential fatty acids, protein, vitamins and minerals, which, taken together, make them an important part of a healthy diet. Used as a nutritious food, many mushroom species are specially collected and used in cooking in many parts of the world. *Hohenbuehelia petaloides* (Bull.) Schulzer (*Pleuroteceae*) is an edible tree mushroom that is naturally distributed in Türkiye. While apoptosis is a programmed cell death, necrosis and autophagy occur when cells are under stress. One of the effective measures for tumor suppression is to reconstruct the signal transduction system of tumor cell apoptosis and induce cancer cell apoptosis. This study aimed to determine whether *H. petaloides* hexane extract (IC_{50}= 93.95± 0.02 µg/mL) induces apoptotic cell death in HT-29 and to determine the amount of cells in the division phase. 300 g dried mushrooms were extracted with hexane then apoptosis and cell cycle analysis were investigated in HT-29 by flow cytometry using ModFit LT according to Yilmazer 2011 method. According to the flow cytometry analysis performed to determine the amount of cells in the division phase, it was determined that the hexane extract of *H. petaloides* induced apoptosis in HT-29 in 24 hours ($2xIC_{50}$ = 42.5%) in parallel with the increase in concentration. Cell cycle analysis, it was found that the number of cells in the G1 phase (IC_{50} concentration 4.42% and 2x IC_{50} concentration 16.76%) increased and the number of cells in the S and G2 phases (IC_{50} concentration 5.41%, and 2x IC_{50} concentration 16.85%) decreased in HT-29 cells at 24 hours as the doses of hexane extract increased compared to the control. In conclusion, *H. petaloides* hexane extract at 2x IC_{50} concentration induces Q2 stage apoptosis in HT-29, leading to the arrest of HT-29 at G1 stage of the cell cycle.

Keywords: *Hohenbuehelia petaloides*, colon cancer cell line, HT-29, apoptosis, cell cycle

INTRODUCTION

While apoptosis is a programmed cell death, necrosis and autophagy occur when cells are under stress. When the apoptosis mechanism is inhibited, excess cells cannot be eliminated by cell death and malignant tumour cells are produced. Therefore, one of the effective measures to suppress tumour is to reconstruct the signal transduction system of tumour cell apoptosis and induce cancer cell apoptosis (Liu et al., 2022). The first application is to analyse the cell cycle by determining the amount of DNA in the cell. The amount of DNA in the cell can be stained with various dyes and the binding of dyes is related to the amount of DNA in the cell. Therefore, flow cytometry can be used to determine the number of cells in each division phase. Diploid cells are in the G0 /G1 phase, while the DNA content of cells in the S (synthesis) phase is between diploid and tetraploid cells. Cells in G2 and Mitosis are observed as tetraploid since they carry DNA in the amount of 4n (Anev and Muranlı 2016). Cells in S+G2+M phase are defined as S Phase Fraction (SPF) and high SPF is an indicator of high proliferation rate (Martinez et al., 1990). Fragmented DNA can be seen in the sub-G1 phase. In the histogram obtained, it is seen to the left of this peak separately from the G0-G1 peak (Kanev and Muranlı., 2016).

Mushrooms are important natural sources of healthy nutrition due to their low calorie content, essential fatty acids, protein, vitamins and minerals. Used as a nutritious food, many mushroom species are specially collected and used in many parts of the world (Duru and Çayan 2015; Park et al., 2024). *Hohenbuehelia petaloides* (Bull.) Schulzer is a member of the *Pleuroteceae* family and is an edible tree mushroom naturally distributed in Türkiye (Sesli et al., 2020). In HT-29, hexane extract showed no toxicity at 24 and 48 hours (IC $_{50}$>200 µg/mL) and showed cytotoxic activity at 72 hours with IC_{50}= 93.95± 0.02 µg/mL (Korkmaz et al., 2024). So that ın this study, flow cytometry analysis was performed to determine whether *H. petaloides* hexane extract (IC_{50}= 93.95± 0.02 µg/mL), which showed cytotoxic effect in HT-29 cell line, induced apoptotic cell death.

MATERIAL AND METHOD

1. Mushroom Material

The edible species *Hohenbuehelia petaloides* (Bull.) Schulzer was freshly collected from *Dichondra pepens* L. and woody tree remains in a mixed forest of *Pinus brutia* L., *Pinus pinea* L. and *Phoenix theophrasti* Greuter trees in Datça Turkey. The fungarium material was deposited in the Natural Products Laboratory of Muğla Sıtkı Koçman University under the code CK010.

2. Mushroom Extract

300 g of dried mushrooms were extracted with hexane under room conditions (24 h x 3 times) system in the solvent system. Solvents were removed in a rotary evaporator.

3. Investigation of Apoptosis By Flow Cytometry

In this study, flow cytometry analysis was performed to determine whether *H. petaloides* hexane extract (IC_{50}= 93.95± 0.02 µg/mL) induced apoptotic cell death in HT-29 cell line (Korkmaz et. al., 2024). The eBioscience™ Annexin V-FITC Apoptosis Detection Kit (Thermo Fisher Scientific, 88–8007-72) was used to determine apoptosis by flow cytometry (Alper and Guneş 2020). HT-29 cells were seeded in 6-well plates at 5×10^5 cells/well. Cells were treated with concentrations of $2xIC_{50}$, IC_{50} and $IC_{50/2}$ values of *H. petaloides* hexane extract. Apoptotis effects of HT-29 cells were analyzed by flow cytometry (BD FASCanto A, BD Biosciences, BD FACSDiva software v6.13). Apoptotic, late apoptotic, viable and necrotic cells were quantitatively determined by flow cytometry.

4. Cell Cycle Analysis by Flow Cytometry

Concentrations of *H. petaloides* hexane extract (IC_{50}= 135.45±0.06 µg/mL) at $2xIC_{50}$, IC_{50} and $IC_{50/2}$ were applied for 24h. Samples were stored at − 20 °C for at least 24 h, then centrifuged at 12,000 rpm for 10 min, and the pellets were washed in cold 5-mL PBS. Cell pellets were then dissolved in PBS containing 0.1% Triton-X. Twenty-microliters of RNase A (200 µg/mL) was added to each pellet and incubated at 37 °C for 30 min. After incubation at room temperature for 15 min, readings were completed using the ModFitLT 30 program related to flow cytometry. According to method Yılmazer 2011, readings were completed using the ModFitLT 30 program related to flow cytometry.

RESULTS AND DISCUSSION

In this study, flow cytometry analysis was performed to determine whether *H. petaloides* hexane extract (IC_{50}= 93.95± 0.02 µg/mL), which showed cytotoxic effect in HT-29 cell line, induced apoptotic cell death. Apoptosis percentages and necrosis were determined by calculating FITC-labelled Annexin V binding and the amount of PI binding to DNA. In addition, cell cycle analysis was performed with flow cytometer to determine the amount of cells in which division phase of the cells. According to in vitro findings, *Pleurotus flabellatus* lectin (IC_{50} ranges of 67 µg/mL) significantly reduced apoptosis induction in HT-29 cell line (Murugesan et al., 2023).

Sherr (1999) reported that the mammalian cell cycle is divided into 4 separate phases: G1, S, G2, and M phases. During G1 phase, cells respond to extracellular signals by either advancing toward another division or withdrawing from the cell cycle into a resting state (G0). Ethanol extract of *Inonotus obliquus* (Chaga mushroom) was reported to cause G1 cell cycle arrest in HT-29 human colon cancer cells (Lee et al., 2015). In the other study the anticancer property of lentinan on colon and gastric cancer cell lines through inhibition of the G2/M phase in the cell cycle has been reported (Park et al., 2024).

In this study according to the results of flow cytometry analysis when analysed Table 1, performed to determine the amount of cells in the division phase, it was determined that the hexane extract of *H. petaloides* induced apoptosis in HT-29 in 24 hours (2xIC_{50} = 42.5%) in parallel with the increase in concentration (Figure1). When analysed cell cycle analysis, it was found that the number of cells in the G1 phase (IC_{50} concentration 4.42% and 2x IC_{50} concentration 16.76%) increased and the number of cells in the S and G2 phases (IC_{50} concentration 5.41%, and 2x IC_{50} concentration 16.85%) decreased in HT-29 cells at 24 hours as the doses of hexane extract increased compared to the control (Figure 2-5). In conclusion, *H. petaloides* hexane extract at 2x IC_{50} concentration induces Q2 stage apoptosis in HT-29, leading to the arrest of HT-29 at G1 stage of the cell cycle.

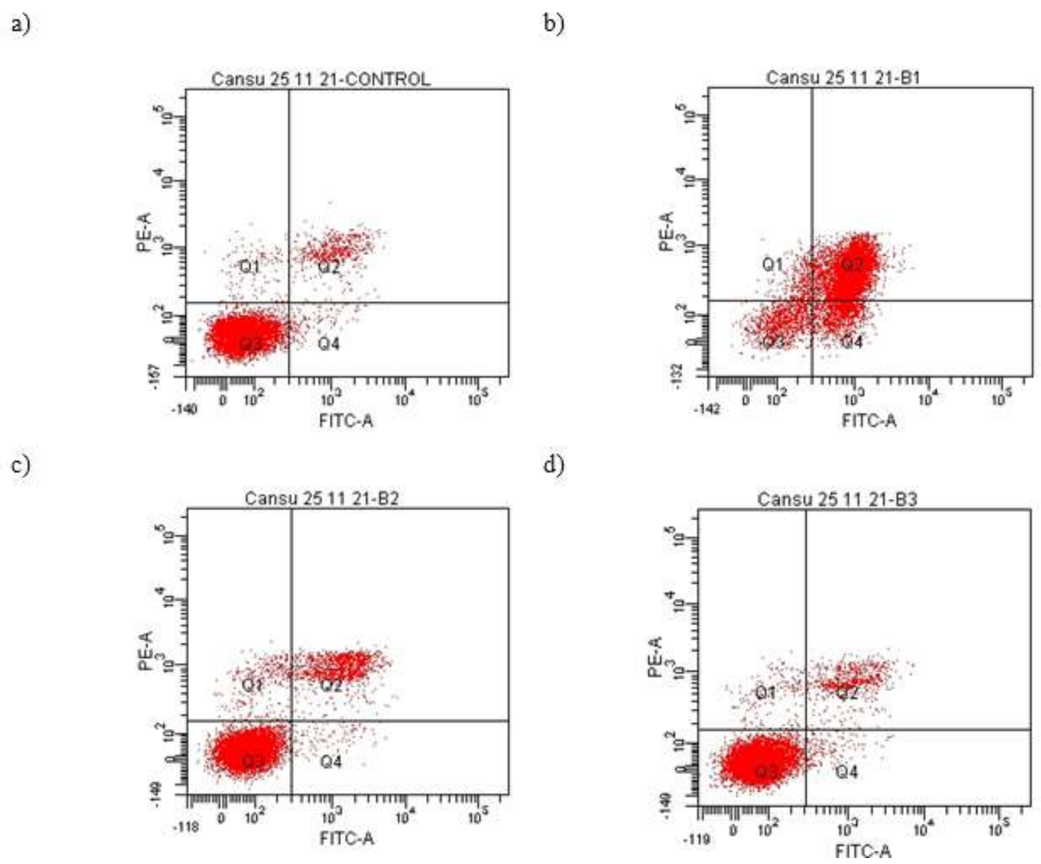


Figure 1. Flow cytometric analysis of HT-29 cells exposed to *H. petaloides* hexane extract concentrations for 24 hours a) control b) 2x IC_{50}: B1 c) IC_{50}: B2 ve d) IC $_{50}$ ½: B3

Table 1. HT-29 cell cycle by ModFit LT programme according to the concentration of *H. petaloides* hexane extract at 2x IC_{50} value

	HT-29 population (%)				
İnkübation time / Extract		Viable Cell (Q3)	Early Apoptosis (Q4)	Late Apoptosis (Q2)	Necrosis (Q1)
24 h / Hexane	**2xIC50**	42.6	7.1	**42.5**	7.8
	IC_{50}	90.4	2.3	6.5	0.8
	IC_{50} 1/2	86	4.7	8.3	1.0
24 h / -	Control	86.9	2.5	10.3	0.3

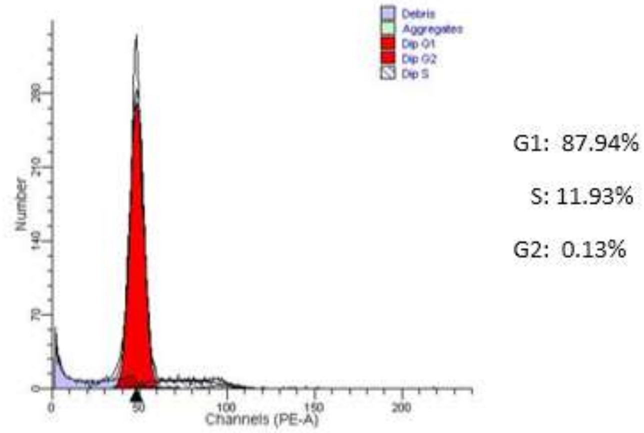


Figure 2. HT-29 cell cycle by ModFit LT programme according to the concentration of *H. petaloides* hexane extract at 2x IC_{50} value

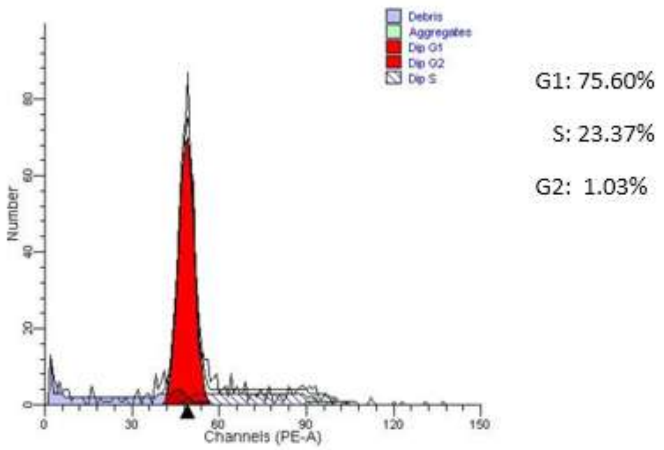


Figure 3. HT-29 cell cycle with ModFit LT programme according to the concentration of *H. petaloides* hexane extract at IC_{50} value

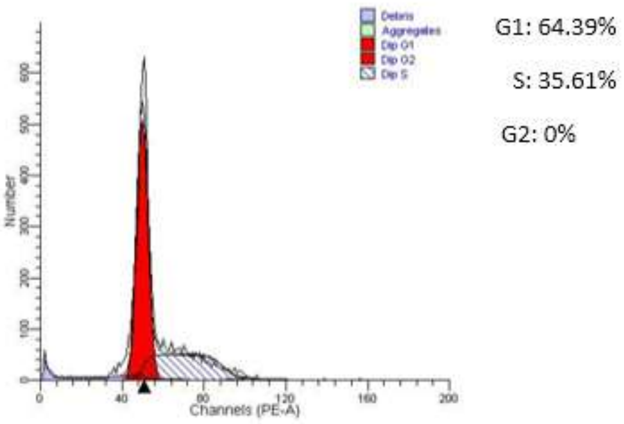


Figure 4. HT-29 cell cycle by ModFit LT programme according to the concentration of *H. petaloides* hexane extract at IC_{50} ½ value

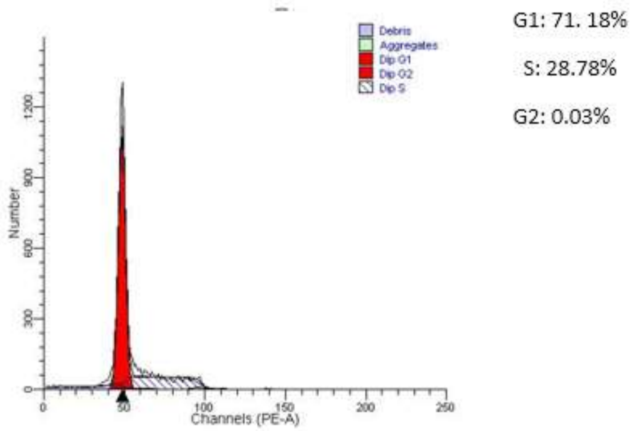


Figure 5. Cell cycle of HT-29 control group with ModFit LT programme

CONCLUSIONS

In conclusion, it was determined that the hexane extract of *H. petaloides* induced apoptosis in HT-29 in 24 hours ($2xIC_{50}$ = 42.5%) in parallel with the increase in concentration. *H. petaloides* hexane extract at 2x IC_{50} concentration induces Q2 stage apoptosis in HT-29, leading to the arrest of HT-29 at G1 stage of the cell cycle. These results shed light on the future for its further use in in vivo and in vitro cancer studies.

ACKNOWLEDGEMENT

This study was supported by Muğla Sıtkı Koçman University Scientific Research Projects Unit (BAP) with the project number 21/129/02/1/4. The authors would like to thank "YÖK 100/2000 Natural and Herbal Products/ Cosmetic" programs for providing PhD scholarship to Cansu Korkmaz. We would also like to thank TUBITAK (BIDEB 2211-A) and BIDEB 2250 projects for their support to Cansu Korkmaz.

REFERENCES

Alper, M. and Güneş, H. 2019. Determination of anticancer effects of Urospermum picroides against human cancer cell lines. International Journal of Secondary Metabolite, 6(1), 28-37.

Alper, M. and Güneş, H. 2020. Cytotoxic Potential of Solanum lycopersicum Leaves Extract on Different Human Cell Lines. Bilecik Şeyh Edebali Üniversitesi Fen Bilimleri Dergisi, 7(2), 544-552.

Duru, M. E., and Çayan, G. T. 2015. Biologically active terpenoids from mushroom origin: a review. Records of Natural Products, 9(4), 456.

J.E. Martinez, J.R. Beck, W.C. Allsbrook, C.G. 1990. Pantazis, Flow cytometric DNA analysis, Clinical Laboratory Science, 3, 180-183.

Kanev, M., and Muranlı, F. G. 2016. Flow sitometri ve kullanım alanları. Sakarya University Journal of Science, 20(1), 33-38.

Korkmaz, C., Güneş, H., Taş Küçükaydın, M., Küçükaydın, S., Duru, M.E. 2024. Biological Activities and Chemical Contents of Edible Hohenbuehelia petaloides (Bull.) Schulzer, ACS Omega, In Press. DOI: 10.1021/acsomega.4c02369.

Lee, H. S., Kim, E. J., & Kim, S. H. 2015. Ethanol extract of Innotus obliquus (Chaga mushroom) induces G1 cell cycle arrest in HT-29 human colon cancer cells. Nutrition research and practice, 9(2), 111-116.

Liu, M. H., Liu, F., Ng, T. B., & Liu, Z. K. 2022. Purification and characterization of pleuroferin, a novel protein with in vitro anti-non-small cell lung cancer activity from the mushroom Pleurotus ferulae lanzi. Process Biochemistry, 122, 16-25.

Murugesan, A. K., Sathuvan, M., & Javee, A. 2023. PFL-lectin regulates the expression of apoptosis-related proteins to antecedent apoptosis in A549 and HT-29 cells. Advances in Cancer Biology-Metastasis, 7, 100099.

Park, G. S., Shin, J., Hong, S., Gopal, J., & Oh, J. W. 2024. Anticarcinogenic potential of the mushroom polysaccharide lentinan on gastric and colon cancer cells: Antiproliferative, antitumorigenic, Mu-2-related death-inducing gene, MUDENG ramifications. Journal of Industrial and Engineering Chemistry, 135, 122-130.

Sesli, E., Asan, A. and Selçuk, F. (edlr.) Abacı Günyar, Ö., Akata, I., Akgül, H., Aktaş, S., Alkan, S., Allı, H., Aydoğdu, H., Berikten, D., Demirel, K., Demirel, R., Doğan, H.H., Erdoğdu, M., Ergül, C.C., Eroğlu, G., Giray, G., Halikî Uztan, A., Kabaktepe, Ş., Kadaifçiler, D., Kalyoncu, F., Karaltı, İ., Kaşık, G., Kaya, A., Keleş, A., Kırbağ, S., Kıvanç, M., Ocak, İ., Ökten, S., Özkale, E., Öztürk, C., Sevindik, M., Şen, B., Şen, İ., Türkekul, İ., Ulukapı, M., Uzun, Ya., Uzun, Yu.,Yoltaş, A. 2020. Türkiye Mantarları Listesi. İstanbul: Ali Nihat Gökyiğit Foundation Publications.

Sherr CJ. Cancer cell cycles. Science. 1996;274:1672–1677.

Yılmazer ÖÇ (2011) DNA fragmentation, cell cycle analysis, apoptotic cell analysis (Annexin V). 1st theoritical cell death research techniques course. Book Chapter ISBN: 978–975–441–349–6, s223–232.

DETERMINATION OF GENETIC DIVERSITY IN SOME MELON VARIETIES USING PEROXIDASE GENE MARKERS

*Seher TOPRAK, Ömer Faruk COŞKUN**

[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Hatay, Türkiye*
**Corresponding author's e-mail: sehertoprak13@gmail.com*

ABSTRACT

Melon is one of the most important summer vegetables produced widely in the world. Many local melon genotyp and commercial varieties are used in cultivation in the world. The success of melon breeding is based on the existence of sources with high genetic diversity, the characteristics of the genetic material and the creation of combinations with the desired characteristics. Genetic characterization of plant material is the most important step of this process. In this study, 14 specific peroxidase gene (POX) primers were used to determine peroxidase gene polymorphism (POGP) in 12 melon genotyp. In the study, 244 of the 259 bands obtained from 14 POX primers were found to be polymorphic. The average polymorphism was 94.4% and the number of bands varied between 13 and 26. The similarity coefficient range of the genotyp varies between 0.30 and 0.90. The genotyp that are genetically closest to each other are number 2 genotyp and number 3 genotyp, while the most distant genotyp are genotyp 1 and 10. The findings obtained from the current study show that there is genetic variation among the melon genotyp examined. The obtained data will enable the melon genotyp with genetic differences to be used more effectively in future breeding programs.

Keywords: *Cucumis melo*, genetic characterization, melon, POGP, POX, UPGMA

INTRODUCTION

Melon is an important cucurbit vegetable that can be produced intensively in tropical and subtropical plants. The origin of melon is probably from Northeast Africa (Swamy, 2017). Melon, which has become an important product in the global market, provides a delicious addition to the cuisines of many countries (Maietti et al., 2012; Manchali et al., 2021). Melon products can cover more than 1 million hectares of land in the world and provide a yield of 28.6 million tons (FAOSTAT, 2022). China ranks first with approximately 14 million tons, and Türkiye ranks second with 1.6 million tons. Melon (*Cucumis melo* L., 2n = 2x = 24), belonging to the Cucurbitaceae family, has a small diploid genome size of 450 Mb (Garcia-Mas et al., 2012). Melon, which has a wide variety and genotypes in the world, shows high levels of features in morphological, characteristic and biochemical properties (Pitrat, 2016).

Changing consumer demands increase the necessity of carrying out new breeding studies in melon. Characterization studies have an important place in a development program of variety. Characterization studies using morphological and biochemical markers are not sufficient due to the effects of the environment. For this reason, the importance of analyzing genomic variation is increasing. DNA sequence differences can be revealed in determining genetic similarities and differences. Marker techniques based on PCR techniques have been used for molecular characterization purposes (Zhang et al., 2016; Karaman et al., 2018; Aslan et al., 2021; Coşkun, 2022). Peroxidase isoenzymes are found in different ratios in many plants. Peroxidases play a role in biotic and abiotic stress conditions in plants (Passardi et al., 2005; Gulsen et al., 2010). Peroxidase gene-based markers (POX) have been used successfully for genetic characterization in some plant species (Gulsen et al., 2010; Uzun et al., 2014).

Genotyping and genetic diversity determination studies can be carried out reliably and rapidly using markers. High-performance genotypes with desired characteristics can be detected in early generations thanks to molecular markers. When the literature is examined, it is seen that genetic characterization studies conducted on melon are insufficient. This study aims to determine the genetic diversity of some melon genotypes using POX markers.

MATERIALS AND METHODS

This study was carried out in the greenhouse and genetic laboratory of the Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University. The melon genotypes previously collected from producers and listed below were used as genetic material.

Table 1. Genotype codes and locations

No	Kodu	Alındığı Yer
1	HMKÜ- KV1	ŞANLIURFA
2	HMKÜ- KV 2	DİYARBAKIR
3	HMKÜ- KV 3	DİYARBAKIR
4	HMKÜ- KV 4	DİYARBAKIR
5	HMKÜ- KV 5	ÇANAKKALE
6	HMKÜ- KV 6	ÇANAKKALE
7	HMKÜ- KV 7	ÇANAKKALE
8	HMKÜ- KV 8	ÇANAKKALE
9	HMKÜ- KV 9	HATAY
10	HMKÜ- KV 10	HATAY
11	HMKÜ- KV 11	HATAY
12	HMKÜ- KV 12	ANTALYA

DNA isolation from young melon leaf samples was performed by the CTAP technique. The operating conditions of the PCR device were optimized as follows. A total volume of 15 µl contained 1.5 µl 10X PCR buffer, 1.33 mM primer, 200 µM each dNTP (dATP, dGTP, dCTP and dTTP), 2.5 mM MgCl2, 1 unit of taq DNA polymerase enzyme, 20 ng DNA and 4.3 µl ddH2O. DNA. Cycling parameters included one cycle of 3 min at 94 ◦C, 34 cycles of 1 min at 94 ◦C, 1 min at 40–53 ◦C, 2 min at 72 ◦C, and for final extension one cycle of 5 min at 72 ◦C. 14 pairs of ISSR primers were used in the studies. The resulting mixture was loaded onto a 2% agarose gel and run for 4 hours under 115 V electric current. After the electrophoresis process, the gels were taken to the gel imaging unit and the gel images were recorded on the computer under UV. Each of the bands displayed on the agarose gel was recorded as ‘’1’’, ‘’0’’ or ‘’9’’, with 1 indicating the presence of the band, 0 indicating the absence of the band, and 9 indicating bands that could not be evaluated. The total number of bands, the number of polymorphic bands, and the polymorphism rates were determined for each primer combination. The obtained data were analyzed in the NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, N.Y.,) USA computer package program. Similarity indices were calculated according to the Dice method and dendrograms were created according to the UPGMA (Unweighted Pair-Group Method With Arithmetic Average) method.

RESULTS AND DISCUSSION

DNA data were obtained in melon genotypes using fourteen pairs of POX markers. In total, 14 pairs of POX primers were used for genetic characterization in 12 melon genotypes. A total of 259 bands and an average of 18.5 bands per primer were obtained. A total of 244 polymorphic bands and an average of 17.43 per primer were determined. The total number of bands per primer varied between 13-26 and the highest number of bands were obtained from the POX-5 primer (26 bands). The number of polymorphic bands in the primers varied between 14-24 and the highest number of polymorphic bands were obtained from the POX-12 primer (24). The polymorphism rate varied between 83.3-100% and the average was calculated as 94.4%. The band range was determined between 110-1640 in all primers.

In this study, it can be evaluated that POX-2, POX-7, POX-8, POX-11, and POX-14 primers are more effective in terms of polymorphism rate in melon genotypes investigated. A 100% polymorphism rate was obtained from these primers. According to the Dice similarity index, similarity coefficient values between genotypes were determined between 0.3007-0.9630. The most distant genotypes are genotype number 1 (genotype taken from Şanlıurfa province) and genotype number 10 (genotype taken from Hatay province) with a similarity coefficient of 0.3007. The closest genotypes are genotypes number 2 and 3 (genotypes taken from Diyarbakır province) with a similarity coefficient of 0.9630.

Table 2. Primer name, primer sequence, numbers of bands, and percentage of polymorphism detected by POX.

Primer Name	Primer Sequences (5’--3’)	Total Number of Bands	Number of Polymorphic Bands	Polymorphism Rate (%)
POX1	5`-CTCGACCTACAAGGAC-3` 5`-ATGTAGGCGCTGGTGA-3`	16	15	93.8
POX2	5`-CTCGACGTCAAGGACCTC-3` 5`-GCCCATCTTCACCATGG-3`	19	19	100.0
POX3	5`-CAACGAGACCAACATCGA-3` 5`-CCTGATCTGTCCCTGCG-3`	13	12	92.3
POX4	5`-TTACGCTACATACAATTTCAA-3` 5`-ACTCGACTGCGACCAG–3`	15	14	93.3
POX5	5`-CACACGATCGGGGCGATC-3` 5`-AATCTGCCGGCAGAGCC-3`	26	23	88.5
POX6	5`-TACCCGACGGTGAGC-3` 5`-CTTGATCGTACTGACTCTA-3`	15	14	93.3
POX7	5`-CTCGACACAACCGATGTTG-3` 5`-TTCACAAACTAGTCACAATCACA-3`	16	16	100.0
POX8	5`-CACCATCAAGAGCGTCATAAC-3` 5`-TTGCTAGAGCGAGCTGG-3`	20	20	100.0
POX9	5`-GGCGTCGGCGTCG-3` 5`-ATCGGGAAGCTTCCCCTC-3`	19	18	94.7
POX10	5`-CCACGCCCTCATCGC-3` 5`-CATCTGGCCGTGCGTC-3`	23	20	87.0
POX11	5`-CCTTCTTCTTGCCATCTTGC-3` 5`-CATATCGCTCCACGACCTTT-3`	16	16	100.0
POX12	5`-CGAGCTGAGAGTGAATCGATC-3` 5`-CTTGAACGCCTGGATGAGC-3`	25	24	96.0
POX13	5`-GACGGTTCTATTGGGAAGAAG-3` 5`-CATGAAAGTGATGAGATGGC-3`	18	15	83.3
POX14	5`-CTCATCGTTAACGTCGCATC-3` 5`-GATGCAAGGAGTATAGTGCAAATG-3’	18	18	100.0
Total		259	244	
Avg		18.5	17.43	94.4

Table 3. Similarity coefficient values between genotypes

	1	2	3	4	5	6	7	8	9	10	11	12
1	1.0000											
2	0.3857	1.0000										
3	0.3611	0.9630	1.0000									
4	0.4144	0.6295	0.6328	1.0000								
5	0.5783	0.4465	0.4545	0.5747	1.0000							
6	0.3230	0.8535	0.8715	0.6520	0.4810	1.0000						
7	0.3291	0.8312	0.8243	0.6966	0.5108	0.9030	1.0000					
8	0.3230	0.8239	0.8235	0.7004	0.4896	0.9118	0.9102	1.0000				
9	0.3571	0.8070	0.8000	0.6967	0.4904	0.8208	0.8306	0.8296	1.0000			
10	0.3007	0.6033	0.6129	0.5909	0.4211	0.6239	0.6293	0.6284	0.6242	1.0000		
11	0.3421	0.8235	0.8301	0.6769	0.4554	0.8421	0.8265	0.8318	0.8299	0.6688	1.0000	
12	0.3429	0.8140	0.8069	0.6721	0.5000	0.7752	0.8106	0.7846	0.7986	0.6242	0.8435	1.0000

In the UPGMA dendrogram, similarity rates vary between 0.46 and 0.96. In the dendrogram, it is seen that genotypes numbered 1 and 5 are clustered separately from the others. Among the remaining genotypes, genotypes numbered 10 and 4 are clustered separately, respectively. It is seen that the remaining 8 genotypes are gathered in a similar cluster. It is possible to state that these genotypes are separated into 3 subclusters. Genotypes numbered 2 and 3 form the first subcluster, genotypes numbered 6, 7, 8 and 9 form the second subcluster and genotypes numbered 11 and 12 form the third cluster. The genotypes clustered closest to each other in the dendrogram are genotypes numbered 2 and 3, as in the Dice similarity index (Figure 1).

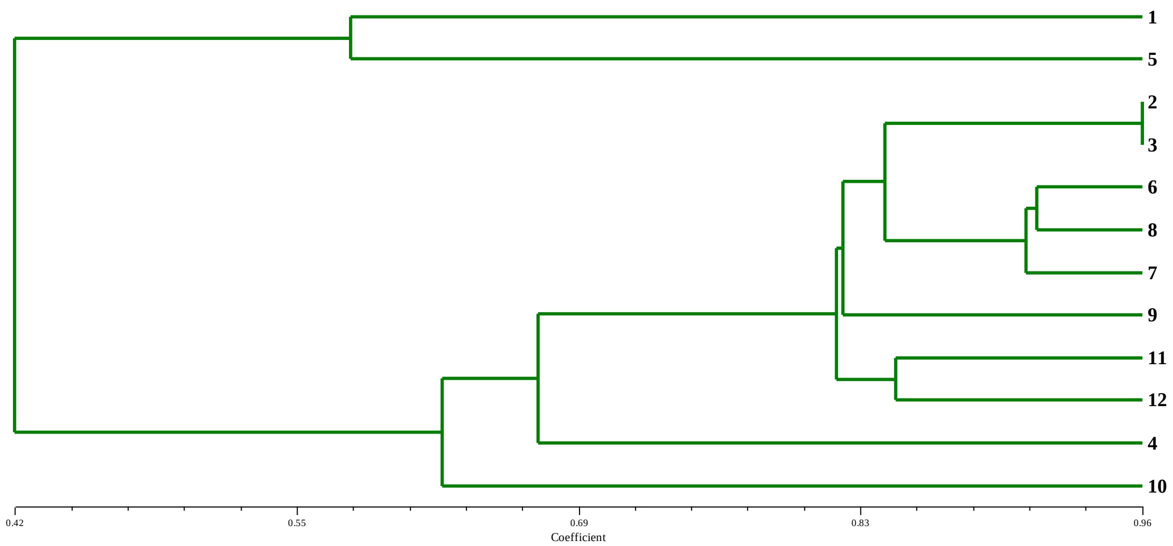


Figure 1. Dendrogram obtained from UPGMA (Unweighted Pair-Group Method with Arithmetic mean) cluster analysis using the Dice coefficient.

Molecular markers are used to direct the genetic potential of plants following breeding purposes. Molecular markers are used in selection, genetic characterization (Toprak et al., 2023), relationship mapping studies (Coskun, 2023a), and genotoxicity studies (2023b). Scorable bands were obtained in all peroxidase gene polymorphism (POGP) primers used in this study. A total of 259 bands were obtained in 12 melon genotypes used with POGP primers.

It was observed that 244 (94.4%) of the 259 bands were polymorphic. Peroxidase gene polymorphism (POGP) bands observed in the investigated melon genotypes showed polymorphism between 83.3% and 100%.

In some previous studies, genetic characterization was determined in melon genotypes. For this purpose, RAPD primers (Nhi et al., 2010), ISSR primers (Guliyev et al. 2018), AFLP primers (Garcia-Mas et al. 2000), SSR primers (Danin-Poleg et al. 2001) were used. In a study, genetic diversity determination study was carried out using 13 SSR markers in melon genotypes and an average of 5.31 alleles per locus was obtained (Solmaz et al., 2016). Yıldız et al. (2011) examined the polymorphism percentage in 63 melon genotypes using different molecular markers and reported that genetic similarities within the group varied between 0.46 and 0.96. In this study, genetic similarity coefficients were determined with POX primers with a wider range (0.3007-0.9630). Chen et al. (2010) determined 265 polymorphic bands in 61 melon genotypes using SRAP primer combinations. Kohpayegani and Behbahani (2008) determined 87% polymorphism in Iranian melon genotypes with 15 SSR markers. In this study, a 94.4% polymorphism rate was obtained using POX primers. In another study, the ISSR marker technique was used in melon genotypes. The polymorphism rate obtained from ISSR primers was 36.3%. Similarity coefficients between genotypes also varied between 0.90 and 0.99 (Coskun and Bozdağ, 2024). When compared with other polymorphism determination studies on melon, it can be said that peroxidase gene polymorphism (POGP) markers are more effective in determining polymorphism in this plant species.

Türkiye is one of the most important countries in the world in terms of plant genetic resources. However, genetic resources are in danger of extinction due to significant changes in ecology, increased monoculture cultivation, irregular construction and human mobility. The protection of these resources is essential to ensure future plant production. For this purpose, it is very important to identify our rich genetic resources. In this study, the genetic characterization of some melon genotypes was determined using the POX marker technique and important data were obtained for breeding studies.

REFERENCES

Aslan, N., Coskun, O. F., Dalda-Sekerci, A., and Gulsen, O. 2021. Moleküler markörler kullanarak çerezlik kabaklarda (*Cucurbita pepo* L.) saflık düzeylerinin tahmin edilmesi. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 26(3): 759-769.

Chen, Y., Li, G., Wang, X.L. 2010. Genetic diversity of a germplasm collection of *Cucumis melo* L. using SRAP markers. Hereditas 32:744-751.

Coskun, O.F., Bozdag, E.A. 2024. Molecular Characterization of some Melon (*Cucumis melo* L.) Genotypes. 16th International İstanbul Scientific Research Congress on Life, Engineering, Architecture, and Mathematical Sciences Proceedings.

Coşkun, Ö. F. 2022. Determination of genetic diversity in some pumpkin genotypes using SSR marker technique. Erzincan University Journal of Science and Technology, 15(3): 942-952

Coşkun, Ö.F. 2023a. Molecular Characterization, Population Structure Analysis, and Association Mapping of Turkish Parsley Genotypes Using iPBS Markers. Horticulturae 2023, 9, 336.

Coşkun, Ö.F. 2023b.The effect of grafting on morphological, physiological and molecular changes induced by drought stress in cucumber. Sustainability 2023, 15, 875.

Danin-Poleg, Y., Reis, N., Tzuri, G. ve Katzir, N. 2001, Development and characterization of microsatellite markers in Cucumis, Theoretical and Applied Genetics, 102 (1), 61-72.

FAOSTAT, 2022. Available online: https://www.fao.org/faostat/en/#data/QCL (accessed on 17 August 2024).

Garcia-Mas, J., Oliver, M., Gomez-Paniagua, H. ve De Vicente, M. 2000. Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon, Theoretical and Applied Genetics, 101 (5-6), 860-864.

Garcia-Mas, J., Benjak, A., Sanseverino, W., Bourgeois, M., Mir, G., González, V.M. 2012. The Genome of melon (*Cucumis melo* L.). Proc. Natl. Acad. Sci., 109, 11872–11877.

Guliyev, N., Sharifova, S., Ojaghi, J., Abbasov, M. ve Akparov, Z. 2018. Genetic diversity among melon (*Cucumis melo* L.) accessions revealed by morphological traits and ISSR markers, Turkish Journal of Agriculture and Forestry, 42 (6), 393-401.

Gulsen, O., Kaymak, S., Ozongun, S., Uzun, A. 2010. Genetic analysis of Turkish apple germplasm using peroxidase gene-based markers. Sci. Hortic. 125, 368–373.

Karaman, K., Dalda-Sekerci, A., Yetisir, H., Gulsen, O., Coskun, O.F. 2018. Molecular, morphological and biochemical characterization of some turkish bitter melon (*Momordica charantia* L.) genotypes. Journal of Industrial Crops and Products, 123: 93-99

Kohpayegani, J.A., Behbahani, M. 2008. Genetic Diversity of some populations of Iranian melon using SSR markers. Biotechnol 7:19-26.

Maietti, A., Tedeschi, P., Stagno, C., Bordiga, M., Travaglia, F., Locatelli, M., Arlorio, M., Brandolini, V. 2012. Analytical traceability of melon (*Cucumis melo* var *reticulatus*): Proximate composition, bioactive compounds, and antioxidant capacity in relation to cultivar, plant physiology state, and seasonal variability. J. Food. Sci., 77, C646–C652.

Manchali, S., Kotamballi, N., Chidambara, M.V., Bhimanagoud, S.P. 2021. Nutritional composition and health benefits of various botanical types of melon (*Cucumis melo* L.). Plants, 10, 1755.

Nhi, P.T.P., Akashi, Y., Hang, T.T.M., Tanaka, K., Aierken, Y., Yamamoto, T., Nishida, H., Long, C., Kato, K. 2010. Genetic diversity in Vietnamese melon landraces revealed by the analyses of morphological traits and nuclear and cytoplasmic molecular markers, Breed Sci, 60 (3), 255-266.

Passardi, F., Cosio, C., Penel, C., Dunand, C. 2005. Peroxidases have more functions than a Swiss army knife. Plant Cell Rep. 24, 255–265.

Pitrat, M. 2016. Melon genetic resources: Phenotypic diversity and horticultural taxonomy. In Genetics and Genomics of Cucurbitaceae; Grumet, R., Katzir, N., Garcia-Mas, J., Eds.; Springer: New York, NY, 1–36.

Solmaz, I., Kacar, Y.A., Simsek, O., Sari, N. 2016. Genetic Characterization of Turkish Snake Melon (*Cucumis melo* L. subsp. melo flexuosus Group) Accessions Revealed by SSR Markers. Biochem Genet., 54(4):534-543.

Swamy, K.R.M. 2017. Origin, distribution and systematics of culinary cucumber (*Cucumis melo* subsp. agrestis var. conomon). J. Hort. Sci., 12, 1–22.

Toprak, S., Coşkun, Ö. F., Mavi, K. 2023. Çerezlik Karpuz Genotiplerinin ISSR Tekniği ile Moleküler Karakterizasyonu. Erciyes Tarım Ve Hayvan Bilimleri Dergisi, 6(1), 51-58.

Uzun, A., Gulsen, O., Seday, U., Yesiloglu, T., Aka Kacar, Y. 2014. Peroxidase gene-based estimation of genetic relationships and population structure among *Citrus* spp and their relatives. Genet. Resour. Crop Evol, 61,1307–1318.

Yildiz, M., Ekbic, E., Keles, D., Sensoy, S., Abak, K. 2011. Use of ISSR, SRAP and RAPD markers to assess genetic diversity in Turkish melon. Sci Hortic 130:349-353.

Zhang, H., Fan, J., Guo, S., Ren, Y., Gong, G., and Zhang, J. 2016. Genetic diversity, population structure, and formation of a core collection of Citrullus accessions. HortScience, 51: 23 -29.

MOLECULAR CHARACTERIZATION OF SOME TOMATO GENOTYPES USING POX TECHNIQUE

Seher TOPRAK, Ömer Faruk COŞKUN*
[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Hatay, Türkiye*
**Corresponding author's e-mail:* sehertoprak13@gmail.com.tr

ABSTRACT

Tomato (*Solanum lycopersicum*) is among the most produced and consumed annual vegetables worldwide. The rich nutritional content of tomato makes it a foodstuff that attracts consumers' attention. Tomato, which is an important foodstuff in terms of both health and economy, is used as a model organism in studies. In this study, 14 specific peroxidase gene (POX) primers were used to determine peroxidase gene polymorphism (POGP) in 12 tomato genotyp. In the study, 127 of the 192 bands obtained from 14 POX primers were found to be polymorphic. The average polymorphism rate was 65.9% and the number of bands varied between 9 and 18. The similarity coefficient range of the genotyp varied between 0.74 and 0.97. According to the UPGMA dendogram, the closest genotyp are determined as the number 7 genotyp and number 8 genotyp, while the most distant genotyp are the number 3 genotyp and number 5 genotyp. The findings obtained from the current study show that there is genetic variation among the domates genotyp examined. The obtained data will enable the domates genotyp with genetic differences to be used more effectively for future breeding programs.

Keywords: Molecular characterization, POX, POGP, *Solanum lycopersicum*, Tomato

INTRODUCTIONS

Tomato (*S. lycopersicum* L.) is one of the most cultivated vegetable species in the world. According to FAO data, tomato production in 2021 had a production capacity of over 189 million tons (FAO, 2024). Tomato fruits are an important source of minerals and vitamins for human nutrition (Tieman et al., 2017). Although tomato production is increasing worldwide, difficulties such as improving quality, tolerance and resistance may arise (Hochholdinger and Baldauf, 2018). To meet the consumption requirement, it is necessary to develop yield, high-quality, disease-resistant tomato varieties (Hickey et al., 2019). Hybrid varieties mostly provide tomato cultivation in the world. Parental characters are of great importance in terms of hybrid variety breeding. Heterosis power mainly depends on the genetic structure and heterogeneity of the parents (Liu et al., 2021). The way to identify different parental characters is to have a good genetic resource pool. In modern cultivated tomatoes, genetic narrowing has increased due to breeding studies (Blanca et al., 2015). Therefore, the characterization of local tomato genotypes may be important for breeding studies. Tomato landraces are intermediate products of the breeding process (Mavromatis et al., 2013).

Determining the genetic structures of plant genotypes is important in plant breeding and new variety development studies. Unlike morphological and biochemical markers, the fact that molecular markers are not affected by environmental conditions is important for obtaining the correct answer. Many marker techniques have been used in genetic characterization studies in vegetables (Karaman et al., 2017; Morilipinar et al., 2021; Coskun, 2023). Genetic characterization studies have been conducted in tomato with PCR-based marker techniques before (Tam et al., 2005; Benor et al., 2008; Aguilera et al., 2011; Ibrahım and Erdinç, 2020). Peroxidase enzymes are important in the stress response of plants. Higher diversity can be found in peroxidase gene sequences among plant genotypes (Zhang et al., 2001). POGP markers have been used to determine genetic diversity in some plant species (Ceylan et al., 2014).

Although tomato is one of the most preferred plant species in genetic studies, the discrimination power of POX primers in tomato is not known sufficiently. Studies conducted to determine molecular diversity of POPG markers in tomato genotypes are not sufficient. The aim of this study is to determine the genetic diversity of 12 different local tomato genotypes using POGP markers.

MATERIALS AND METHODS

Twelve tomato genotypes collected from Hatay and its surroundings were used as material (Table 1). DNA was isolated from young tomato leaves according to the CTAB protocol (Doyle and Doyle, 1987). DNA quantity and quality were determined by agarose gel method. 14 pairs of POPG primers were used in this study (Table 2).

Table 1. Hatay Tomato Genotype Information

No	Code	Location
1	HMKÜ- DO1	SAMANDAĞ
2	HMKÜ- DO 2	SAMANDAĞ
3	HMKÜ- DO 3	SAMANDAĞ
4	HMKÜ- DO 4	SAMANDAĞ
5	HMKÜ- DO 5	SAMANDAĞ
6	HMKÜ- DO 6	SAMANDAĞ
7	HMKÜ- DO 7	SAMANDAĞ
8	HMKÜ- DO 8	SAMANDAĞ
9	HMKÜ- DO 9	ALTINÖZÜ
10	HMKÜ- DO 10	ALTINÖZÜ
11	HMKÜ- DO 11	ALTINÖZÜ
12	HMKÜ- DO 12	ALTINÖZÜ

The reaction mixture (15 ml) contained 30 pmol of each primer pairs, 200 mM of each of dNTPs, 2.5 mM of MgCl2 as a final concentration, 1 unit of Taq polymerase and 25 ng of template DNA. PCR amplification included a denaturing stage at 94 ˚C for 2 minutes, followed by 34 cycles denaturing step at 94 ˚C for 1 minutes, an annealing step at a temperature according the melting temperature of each primer for 2 min and an extension step at 72 ˚C for 2 minute, one cycle of 5 min at 72 C. PCR products were separated on 2 % agarose gel at 110 V for 5 h, stained with Ethidium Bromide and visualized and 1, 0 and 9 data were obtained. Clustering analyzes were performed using NTSYS (Numerical Taxonomy Multivariate Analysis System, NTSYS-pc version 2.1, Exeter Software, Setauket, N.Y., USA) package program (Rohlf, 2000).

Similarity indexes between individuals were calculated according to the DICE method (Dice, 1945). UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram based on DICE similarity matrix.

RESULTS AND DISCUSSION

In this study, the effectiveness of some POPG markers in local tomato genotypes was determined and genetic characterization study was carried out. In the study carried out with fourteen pairs of POPG primers, a total of 192 bands were obtained and 127 of them were found polymorphic. The polymorphism rate varied between 26.7% and 100% and the average was determined as 65.9% (Table 2).

Table 2. Polymorphism and Band Information Obtained from POX primers

Primer Name	Primer Sequences (5’--3’)	Number of Polymorphic Band	Number of Total Band	Polymorphism Rate (%)
POX1	5\`-CTCGACCTACAAGGAC-3\` 5\`-ATGTAGGCGCTGGTGA-3\`	5	14	35.7
POX2	5\`-CTCGACGTCAAGGACCTC-3\` 5\`-GCCCATCTTCACCATGG-3\`	13	16	81.3
POX3	5\`-CAACGAGACCAACATCGA-3\` 5\`-CCTGATCTGTCCCTGCG-3\`	4	9	44.4
POX4	5\`-TTACGCTACATACAATTTCAA-3\` 5\`-ACTCGACTGCGACCAG–3\`	13	13	100.0
POX5	5\`-CACACGATCGGGGCGATC-3\` 5\`-AATCTGCCGGCAGAGCC-3\`	6	14	42.9
POX6	5\`-TACCCGACGGTGAGC-3\` 5\`-CTTGATCGTACTGACTCTA-3\`	4	15	26.7
POX7	5\`-CTCGACACAACCGATGTTG-3\` 5\`-TTCACAAACTAGTCACAATCACA-3\`	9	10	90.0
POX8	5\`-CACCATCAAGAGCGTCATAAC-3\` 5\`-TTGCTAGAGCGAGCTGG-3\`	3	9	33.3
POX9	5\`-GGCGTCGGCGTCG-3\` 5\`-ATCGGGAAGCTTCCCCTC-3\`	13	14	92.9
POX10	5\`-CCACGCCCTCATCGC-3\` 5\`-CATCTGGCCGTGCGTC-3\`	12	18	66.7
POX11	5\`-CCTTCTTCTTGCCATCTTGC-3\` 5\`-CATATCGCTCCACGACCTTT-3\`	12	12	100.0
POX12	5\`-CGAGCTGAGAGTGAATCGATC-3\` 5\`-CTTGAACGCCTGGATGAGC-3\`	13	16	81.3
POX13	5\`-GACGGTTCTATTGGGAAGAAG-3\` 5\`-CATGAAAGTGATGAGATGGC-3\`	7	17	41.2
POX14	5\`-CTCATCGTTAACGTCGCATC-3\` 5\`-GATGCAAGGAGTATAGTGCAAATG-3’	13	15	86.7
Total		127	192	
Mean		9.07	13.71	65.9

The number of polymorphic bands varied between 3 and 13 and the average was determined as 9.07. The total number of bands varied between 9 and 18 and the average was determined as 13.71. The highest total number of bands was obtained from POX10 primer (18), the lowest total number of bands was obtained from POX8 and POX3 primers (9). The lowest number of polymorphic bands was obtained from POX8 primer (3), and the highest number of polymorphic bands was obtained from POX2, POX4, POX9, POX12 and POX14 primers (13). While the lowest polymorphism rate was determined in POX6, the highest polymorphism rate was obtained from POX4 and POX11 primers with 100% (Table 2).

The similarity coefficients determined according to the DICE similarity index using the NTSYS program were determined between 0.74 and 0.94. The closest genotypes to each other are genotypes 7 and 8 with a similarity coefficient of 0.97. Also, genotypes 7 and 9 are the closest to each other with a similarity coefficient of 0.97. The farthest genotypes to each other are genotypes 5 and 6 with a similarity coefficient of 0.74.

Table 3. Similarity coefficient values among tomato genotypes according to dice similarity index

	1	2	3	4	5	6	7	8	9	10	11	12
1	1.0											
2	0.93	1.0										
3	0.87	0.91	1.0									
4	0.91	0.93	0.88	1.0								
5	0.77	0.81	0.76	0.76	1.0							
6	0.85	0.88	0.80	0.88	0.74	1.0						
7	0.92	0.94	0.87	0.91	0.83	0.90	1.0					
8	0.90	0.92	0.86	0.90	0.81	0.89	0.97	1.0				
9	0.91	0.93	0.86	0.91	0.82	0.89	0.97	0.96	1.0			
10	0.89	0.90	0.82	0.90	0.75	0.88	0.92	0.91	0.93	1.0		
11	0.91	0.92	0.83	0.87	0.80	0.84	0.92	0.92	0.93	0.90	1.0	
12	0.91	0.91	0.83	0.91	0.77	0.88	0.92	0.92	0.92	0.94	0.93	1.0

In the UPGMA dendrogram made according to the DICE similarity index, 3 main clusters were formed. The first cluster included genotypes 1, 2 and 4; the second cluster included genotypes 7, 8, 9, 10, 11 and 12. Genotypes 6, 3 and 5 were clustered further away from these genotypes, respectively. In the clustering analyses, it was determined that genotype number 5 was separated from the others with a similarity rate of 0.78.

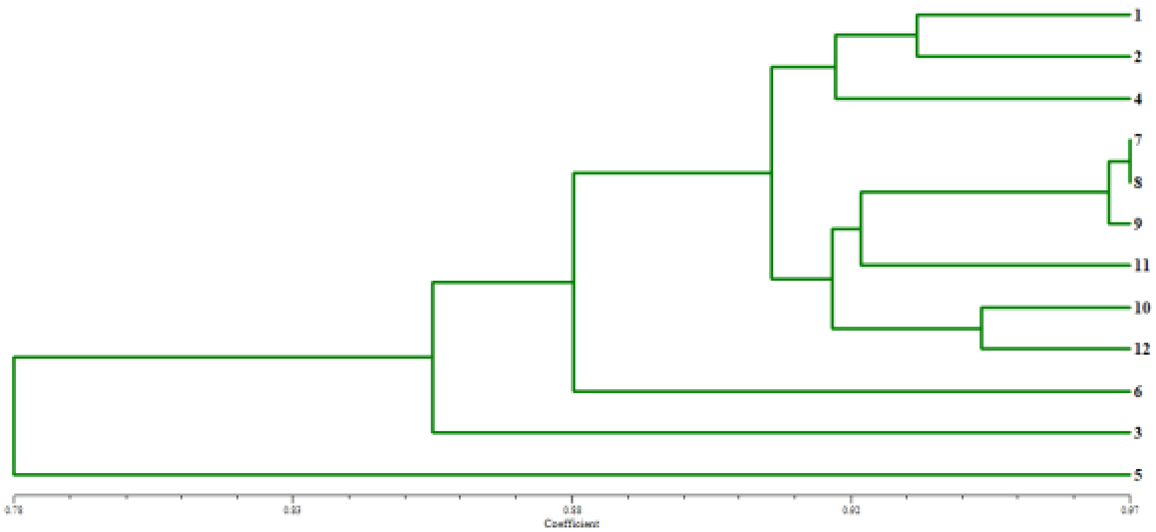


Figure 1. Dendrogram obtained by using the similarity indices obtained from POPG data in the SHAN module.

Many molecular studies have been conducted on tomatoes in the world and in Türkiye, and tomatoes have been used as a model plant. Tomatoes are grown locally in many cities in Türkiye. After long-term cultivation, the number of local populations has increased. Determining the characteristics of genetic resources is important for the preservation of genetic resources and their use in breeding. Hatay is an important city where local tomato genotypes are found. Studies determining the genetic differences of tomato genotypes collected from this city are few. Determining the genetic structure of Hatay tomato genotypes is important for breeding studies. The genetic diversity study in Hatay tomato genotypes was previously determined using the ISSR marker technique (Coskun and xxxx., 2024). With this study, the effectiveness of POX primers was determined for the first time in this material.

Villand et al. (1998) determined the genetic structure among 96 genotypes by obtaining 98 polymorphic RAPD markers from 41 RAPD primers. Benor et al. (2008) detected a total of 150 alleles using 35 different SSR polymorphic markers to determine the genetic diversity of 39 tomato lines. Kwon et al. (2009) obtained 132 polymorphic bands with 33 SSR markers in 63 different commercial tomato cultivars. Kaushal et al. (2017) detected 22 SSR alleles using twenty different SSR markers. Castellana et al. (2020) obtained 60 alleles using SSR markers in 121 tomato genotypes. In his study, Öztürk (2022) conducted a genetic characterization study using the ISSR technique on 24 tomato genotypes and determined the average polymorphism rate as 80%. In this study, an average polymorphism rate of 65.9% was detected with POX primers. In a study, the genetic similarity coefficient between tomato genotypes was determined between 0.56 and 0.95 using the ISSR marker technique (Terzopoulos and Bebeli, 2008). In the study conducted by Al-Shaal et al. (2021), the average genetic similarity coefficient in tomato genotypes was determined as 0.79 using ISSR markers. In this study, the genetic similarity coefficient was determined between 0.74 and 0.94.

When the literature was examined, it was determined that the use of SSR markers from PCR techniques in tomatoes has increased in recent years and that they can distinguish polymorphism. In this study, it was determined that genetic diversity can be distinguished with POX primers in tomatoes. In this study, the polymorphism rate was found to be similarly high. Local tomato genotypes are grown in many locations in Türkiye. It is important to characterize tomato genotypes with marker techniques. Different marker techniques may be more advantageous in genetic characterization. As a result of this study, it can be concluded that there is genetic diversity among tomato genotypes and that the POPG marker technique can be used due to its ability to show polymorphism. The results of this study provide important information to improve the characteristics of new varieties in tomato breeding programs.

REFERENCES

Aguilera, J. G., Pesson, L. A., Rodrigues, G. B., Elsayed, A.Y., da Silva, D. J. H., de Barros, E. G. 2011. Genetic variability by ISSR markers in tomato (*Solanum lycopersicon* Mill.). Revista Brasileira de Ciências Agrárias, 6(2): 243-252.

Al-Shaal A, Murshed RA, Albiski FA 2021. Molecular charac-terization of some tomato genotypes using inter simple sequencerepeats (ISSR) technique. J Genet Environ 9(1):134–142

Benor, S., Zhang, M., Wang, Z., & Zhang, H. 2008. Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. Journal of Genetics and Genomics, 35(6), 373-379

Blanca, J., Montero-Pau, J., Sauvage, C., Bauchet, G., Illa, E., Díez, M.J., Francis, D., Causse, M., van der Knaap, E., Cañizares, J. 2015. Genomic variation in tomato, from wild ancestors to contemporary breeding accessions. *BMC Genom. , 16*, 257.

Castellana, S., Ranzino, L., Beritognolo, I. et al. 2020. Genetic characterization and molecular fingerprint of traditional Umbrian tomato (*Solanum lycopersicum* L.) landraces through SSR markers and application for varietal identification. Genet Resour Crop Evol 67, 1807–1820.

Ceylan, A., Öcal, N., Akbulut, M. 2014. Genetic diversity among the Turkish common bean cultivars (*Phaseolus vulgaris* L.) as assessed by SRAP, POGP and cpSSR markers. Biochemical Systematics and Ecology, 54, 219–229.

Coşkun, Ö.F. 2023. Molecular characterization population structure analysis and association mapping of Turkish parsley genotypes using iPBS markers. Horticulturae, 9 (3), 336. https://doi.org/10.3390/horticulturae9030336

Coskun, O.F., Durmuş A.N. 2024. Determination of genetic divercity of Hatay tomato genotypes by ISSR technique. 16th International İstanbul Scientific Research Congress on Life, Engineering, Architecture, and Mathematical Sciences Proceedings.

Dice, L.R. 1945. Measures of the amount of ecologic association between species. Ecology, 26, 297-302.

Doyle, J. J., & Doyle, J. L. 1987. A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochemical Bulletin, 19, 11-15.

Hickey, L.T., Hafeez, A.N., Robinson, H., Jackson, S.A., Leal-Bertioli, S.C.M., Tester, M., Gao, C., Godwin, I.D., Hayes, B.J., Wulff, B.B.H. 2019. Breeding crops to feed 10 billion. *Nat. Biotechnol.*, *37*, 744–754.

Hochholdinger, F., Baldauf, J.A. Heterosis in plants. *Curr. Biol.* 2018. *28*, 1089–1092.

İbrahım, A. S., Erdinç, Ç. 2020. Determination of genetic relations among tomato accessions in sulaymaniyah region through ISSR markers genetic relations in tomato accessions. Yuzuncu Yıl University Journal of Agricultural Sciences, 30(4), 810-820.

Karaman, K., Dalda-Sekerci, A., Yetisir, H., Gulsen, O., and Coskun, O. F., 2018. Molecular, morphological and biochemical characterization of some turkish bitter melon (*Momordica charantia* L.) genotypes. Jou

Kaushal, A., Singh, A. and Jeena, A.S., 2017. Genetic diversity in tomato (*Solanum lycopersicum* L.) genotypes revealed by simple sequence repeats (SSR) markers, Journal of Applied and Natural Science, 9, 966-973

Kwon, Y., Park, S. and Yi, S., 2010. Assessment of genetic variation among commercial tomato (*Solanum lycopersicum* L) varieties using SSR markers and morphological characteristics, Genes & Genomics, 31, 1-10.

Liu, Z., Jiang, J., Ren, A., Xu, X., Zhang, H., Zhao, T., Jiang, X., Sun, Y., Li, J., Yang, H. 2021. Heterosis and combining ability analysis of fruit yield, early maturity, and quality in tomato. Agronomy , 11, 807.

Mavromatis A.G., Athanasouli V., Vellios E., Khah E., Georgiadou E.C., Pavli O.I. 2013. Arvanitoyannis I.S. Characterization of tomato landraces grown under organic conditions based on molecular marker analysis and determination of fruit quality parameters. J. Agric. Sci. 5:239–252.

Morilipınar, E.O., Dalda Ş., A., Coşkun, Ömer F., Gülşen, O. 2022. Genetic analysis of local pumpkin populations. International Journal of Agricultural and Natural Sciences, 14(3), 264–272

Öztürk, H.İ. 2022. Morphological and molecular characterization of some Tomato (*Solanum lycopersicum* L.) genotypes collected from Erzincan Province of Türkiye. Mol Biol Rep 49, 7111–7121.

Rohlf, J.F. 2000. NTSYS-pc: Numerical Taxonomy And Multivariate Analysis System. Exeter Software, Setauket, New York

Tam, S. M., Mhiri, C., Vogelaar, A., Kerkveld, M., Pearce, S. R., Grandbastien, M. A. 2005. Comparative analyses of genetic diversities within tomato and pepper collections detected by retrotransposon-based SSAP, AFLP and SSR. Theoretical and Applied Genetics, 110(5), 819-831

Terzopoulos PJ, Bebeli PJ. 2008. DNA and morphological diver-sity of selected Greek tomato (*Solanum lycopersicum* L.) landra-ces. Sci Hortic 116:354–361

Tieman D., Zhu G., Resende M.F.R., Jr., Lin T., Nguyen C., Bies D., Rambla J.L., Beltran K.S.O., Taylor M., Zhang B., et al. 2017. A chemical genetic roadmap to improved tomato flavor. Science.;355:391–394.

Villand, J.M., Skroch, P.W., Lai, T.V., Hanson, P., Kuo, C.G. and Nienhuis, J., 1998. Genetic Variation among Tomato Accessions from Primary and Secondary Centers of Diversity, Crop Science, 38, 1339-1347.

Zhang, L., S.K. Pond, B.S. Gaut. 2001. A survey of the molecular evolutionary dynamics of twenty-five multigene families from four taxa. J. Mol. Evol., 52, pp. 144-156

GROWTH, MORTALITY AND YIELD OF OHRID TROUT STOCK IN LAKE OHRID

Valbona KOLANECI[1], Marsida BLLACA[1]

Agricultural University of Tirana, Faculty of Agriculture and Environment Department of Aquaculture and Fisheries, Tirana, Albania

Corresponding author e-mail: vkolaneci@ubt.edu.al

ABSTRACT

The fish fauna of Lake Ohrid stands out as one of Europe's most diverse and abundant. This ancient lake harbors seventeen native fish species, among them the endemic Ohrid trout (*Salmo letnica*) and belushka/belvica (*Salmo ohridanus*). In 2022, inland waters fishery production in Albania totaled 3,388 tons, with Lake Ohrid contributing an estimated 155.5 tons. Of this, 93 tons were Ohrid trout catch. While the Lake's production may seem modest compared to Albania's total fishery production of 17,799 tons (including marine, inland waters, and aquaculture) for the same year, it is of significant local importance. The fishery constitutes the primary source of income for many families in the fishing villages along the Albanian part of Ohrid lakeshore. Despite the control measures implemented over the past decade to manage the exploitable fish stocks of Lake Ohrid, the lake's fish populations have faced various stressors, including pollution, habitat degradation, and invasive species, in addition to overfishing. Furthermore, the Ohrid trout is being exploited without sufficient knowledge of its stock status. The IUCN Red List of Threatened Species lists the Ohrid trout as Data Deficient. This study aims to provide an initial assessment of the condition and productivity of the Ohrid trout stock in Lake Ohrid. Growth parameters were calculated using length at age analysis of fish collected by the local small-scale fishery, while the total mortality rate was estimated through length-converted catch curve analysis. Stock production was predicted using the model developed by Beverton and Holt and adapted for FiSAT II (2005). The growth of the Ohrid trout population was expressed using the total length-based function L_t=68.0*(1-$e^{-0.21*(t+0.01)}$). The total mortality rate was estimated at M=0.76 yr^{-1} and the exploitation rate at E=0.51 yr^{-1}. The model's predicted production indicates that the Ohrid trout stock is currently exploited at maximum sustainable yield. Maintaining the same exploitation rate while increasing the length at first capture to 32 or 35 cm would increase the stock's relative yield per recruit (Y'/R) by 0.035. Implementing the national fishery regulation on minimum size at first catch for Ohrid trout would not only ensure sustainable productivity but also support population growth.

Keywords: Ohrid trout, growth, mortality, stock.

INTRODUCTION

Lake Ohrid, located on the border between Albania and North Macedonia, is a significant freshwater lake renowned for its natural values, large number of habitats, rich biodiversity and its indisputable cultural and historical assets. The Lake is home is home to indigenous species, including its trout species (*Salmo letnica* and *Salmo ohridanus*) which are key components of its unique aquatic ecosystem and making it a critical site for trout fisheries in the region (Spirkovski & Talevski 2002, Spirkovski et al., 2017; Bllaca & Kolaneci, 2023). Trout fishery is an important economic activity for the communities around Lake Ohrid, offering a significant source of income and sustaining the livelihoods of many families, especially those residing in the fishermen villages along the lakeshore.

In 2022, inland waters fishery production in Albania totaled 3,388 tons, with Lake Ohrid contributing an estimated 155.5 tons (INSTAT, 2023). Of this, 93 tons were Ohrid trout (Bllaca & Kolaneci, 2023). While the Lake's production may seem modest compared to Albania's total fishery production of 17,799 tons (including marine, inland waters, and aquaculture) for the same year (INSTAT, 2023), it is of significant local importance. The fishery constitutes the primary source of income for many families in the fishing villages along the Albanian part of Ohrid lakeshore. The fishery activities within the Albanian border of Lake Ohrid are typically family-operated. There are 220 boats, with engines ranging from 3.5 to 15 horsepower, holding fishing permits. The primary fishing gear used includes nets (ranging from 500 to 1000 meters in length, with mesh sizes from 26-32 mm to 45-90 mm) and hook lines (50 meters in length with up to 150 hooks) (Bllaca & Kolaneci, 2023). To support the Ohrid trout fishery, the Lake is regularly stocked with fingerlings. This supplementation aims to maintain or increase trout populations, counteracting the impacts of fishing and natural mortality (Spirkovski et al., 2017, INSTAT, 2023). However, there is no monitoring in place to ensure that the stocking and fishing practices do not exceed the lake's ecological capacity.

Despite the implementation of control measures over the past decade to manage the exploitable fish stocks in Lake Ohrid, the lake's fish populations are facing various stressors. These include pollution, habitat degradation, invasive species, and overfishing (Kostoski et al., 2010; Spirkovski et al., 2017; Schöffmann & Marić, 2023). These studies emphasizes that the population structure and health of Ohrid Trout are crucial indicators of the lake's overall ecological status. Moreover, the exploitation of Ohrid trout in Lake Ohrid occurs without adequate knowledge of the stock's status. The IUCN Red List of Threatened Species classifies the Ohrid trout as "Data Deficient" (Crivelli, 2006), highlighting the urgent need for uptodate and reliable data on stock structure, abundance and dynamic in order to assess and manage the population effectively. This study aims to provide an initial assessment of the condition and yield of the Ohrid trout stock in Lake Ohrid.

MATERIAL AND METHOD

Fish samples collected from local small-scale fishery on the main landing sites of fishing boats along the Albanian part of Lake Ohrid shore (Figure 1a). Ohrid trout were fished within the Albanian border of the Lake area, using seine fishing of 1000 m length and 45 mm mesh size and hook lines with up to 150 hooks. The sampling, adapted after Spare & Venema (1992), took place in four subsequent days in June 2023. The total length (TL) data were composed together as a single time collection Taking in consideration the short period of sampling, the fish growth within sampling period was not taken in consideration. The total length (TL) of 89 sampled individuals was measured up to 0.1 cm and grouped into 1 cm length classes. for each length class, the average age was calculated through and the age were estimated by scale reading (Figure 1b). The estimation of growth parameters of Ohrid trout population was performed based on length at age analysis (direct search using nonlinear method of least squares) that involve a direct search of the values of K, L_∞ and t_0, that best fit a curve through the length at age data (Gayanilo et al., 2005). The analysis of pseudo cohorts was performed to obtain the growth function of the population.

Figure 1. (a) Primary landing sites of fishing boats, where fish samples were collected (Google Maps 2023); (b) Measurement of Ohrid trout lengths.

Total mortality (Z) for Ohrid trout population was estimated through length-converted catch curve (Pauly 1984; 1990). Natural mortality (M) was estimated according to Pauly's empirical formula $\ln M=-0.0152-0.279*\ln L_\infty+0.6543*\ln K+0.463*\ln T$ (Pauly, 1980) for the average annual temperature of Lake Ohrid of $T=13.7^0C$. Fishing mortality (F) was estimated as $F=Z-M$, while the exploitation rate (E) as $E=F/Z$.

Prediction of the stock production as relative yield per recruit (Y'/R) was evaluated after the model developed by Beverton and Holt (1966) and adapted for FiSAT II (Gayanilo et al., 2005). The calculations were done using the knife edge method and the values of the ratio L_c/L_∞ and M/K. The biological target reference values of exploitation were compared with the current exploitation rate and utilized to assess the status of the Ohrid trout stock in Lake Ohrid.

RESULTS AND DISCUSSION

The sample of Ohrid trout consisted of 89 individuals, with total lengths (TL) ranging from 25.0 to 51.7 cm. The most common age groups among the sampled fish were 3 and 4 years, corresponding to mean total lengths of 31.86 cm and 38.71 cm, respectively. The growth parameters for Ohrid trout were estimated as follows: asymptotic length (L_∞) of 68.0 cm (TL), growth coefficient (K) of 0.21 yr^{-1}, and age at length zero (t_0) of -0.01yr. The growth of the Ohrid trout population was described using the following total length-based function: $L_t=68.0*(1-e^{-0.21*(t+0.01)})$ (Figure 2). According to Kottelat & Freyhof (2007), the maximum standard length (SL) for Ohrid trout in Lake Ohrid can reach up to 60 cm. Additionally, a maximum total length of 76.0 cm has been reported for introduced Ohrid trout in some lakes and reservoirs in Colorado, Minnesota, Montana, Tennessee, and Wyoming, USA (Crawford, 1993).

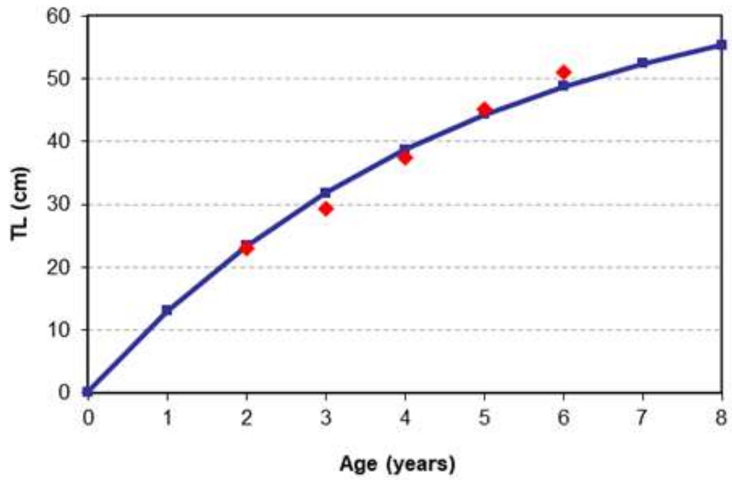


Figure 2. Von Bertalanffy growth curve for Ohrid trout population (red dots: observed values, blue dots: calculated values).

The total mortality rate (Z) for the Ohrid trout population was calculated to be 0.76 yr^{-1}. Natural mortality (M) and fishing mortality (F) were estimated at 0.37 yr^{-1} and 0.39 yr^{-1}, respectively. The exploitation rate (E), which represents the proportion of the total mortality rate attributable to fishing, was estimated at E=0.51 yr^{-1}. For decades, Lake Ohrid has been stocked with Ohrid trout fingerlings by the respective fishery authorities of Albania and North Macedonia. Although not empirically tested, it is likely that this additional recruitment compensates for the higher fishing mortality rate, helping to maintain the population size and structure.

The Relative Yield per Recruit Analysis (Y'/R) for the Ohrid trout stock in Lake Ohrid is presented in Figure 3. With a current stock exploitation rate of E=0.51 yr^{-1} and parameter values of M=0.37 yr^{-1}, K=0.21 yr^{-1}, L_∞=68.0 cm and L_c=20.4 cm, the relative yield per recruit of the current stock is Y'/R=0.027 (indicated by the red star on the isobars in Figure 3a). A simulation was conducted to examine the effect of increasing the length at first capture (L_c) on relative yield (Figure 3b). The model indicates that increasing L_c to 32 or 35 cm, while maintaining the current exploitation rate of E=0.51 yr^{-1}, would result in a relative yield per recruit of Y'/R=0.035 (represented by the red star on the isobars in Figure 3b). Additionally, along with increasing L_c, reducing the exploitation rate below E=0.51 yr^{-1} would not only sustain the stock's productivity but also support its growth.

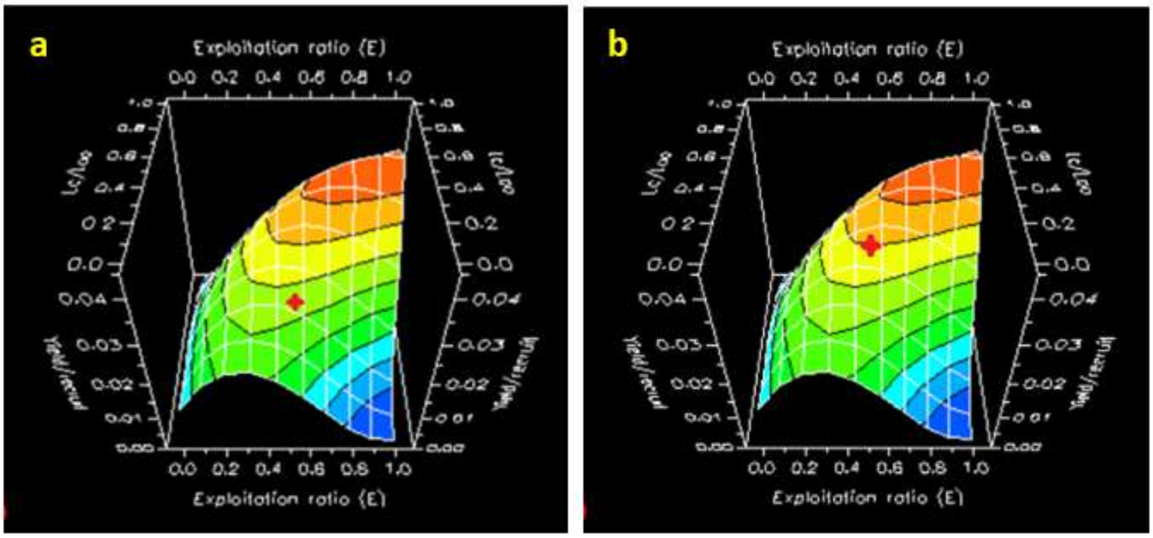


Figure 3. Relative Yield per Recruit Analysis (Knife-edge Selection) for the Ohrid trout stock as a function of the exploitation rate (E) and the ratio of length at first capture to asymptotic length (L_c/L_∞) (a) Y'/R of the stock (indicated by the red star on the isobars) for E=0.51 yr^{-1} and L_c=20.4 cm. (b) Y'/R of the stock (indicated by the red star on the isobars) for E=0.51 yr^{-1} and L_c=32.0 cm.

It should be noted that the low calculated value of L_c results from fishing trout below the minimum allowable size, which is also evident in the sample. If the regulation (MoARD, 2022) enforcing a minimum allowable size of 32 cm for the species had been strictly applied, as simulated in Figure 3b, the stock's production under the current level of exploitation would have been higher.

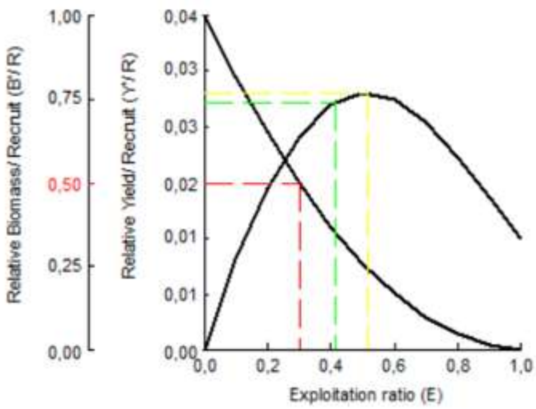


Figure 3. Production prediction as Y´/R and B´/R, as function of exploitation rate (E) for Ohrid trout stock.

The rate of exploitation rate that is currently applied to the trout reserve (E=0.51 yr-1) is almost equal to the rate of exploitation that ensures the maximum sustainable yield per recruit to the trout stock (E_{max}=0.515 yr^{-1}) (indicated by the yellow line in Figure 4). If a more conservative approach is adopted, the exploitation rate should be reduced to the E_{10} level of 0.413 yr^{-1}. This reduction ensures that the stock remains well within safe limits, avoiding overfishing (indicated by the green line in Figure 4). The long-term conservation objective for the stock can be reached with the reduction of exploitation rate at E_{50}=0.300 yr^{-1}. In long term it would provide a biomass level equal to the half of the initial population biomass (indicated by the red line in Figure 4).

It should be noted that data on the population dynamics and stock levels of the Ohrid trout over the past decades are lacking, making it impossible to predict trends in population abundance. The stocking of the lake with trout fingerlings (approximately 1.6 million fingerlings were stocked by the Albanian Fishery Authority in 2022, INSTAT, 2023) is not supported by reliable data on the current stock status or the impact the stocking has on the population. The information provided in this paper are limited and represents only an initial step in a broader study aimed at evaluating the dynamics of the populations and the status of native trout stocks in Lake Ohrid.

CONCLUSIONS

High fishing mortality rate of Ohrid trout stock while the exploitation rate actually applied is at the level of Maximum Sustainable Yield (MSY) might be consequence of yearly supplementing the stock with fingerlings. In such a scenario, careful management and monitoring are crucial to ensure that the combined impacts of fishing and natural mortality do not surpass the stock's recruitment capacity. To evaluate whether natural recruitment combined with supplemental stocking is sufficient to sustain the population and yield at MSY despite high fishing mortality, the future studies should include the recruitment analysis of the stock. For the Ohrid trout stock, it is recommended to operate with an exploitation rate below the E_{max} level. This precautionary measure takes into account the limited area of the species' distribution, as well as uncertainties and variability in stock dynamics. It is advisable for the Ohrid trout stock to maintain an exploitation rate below the E_{max} level. This precautionary approach considers the species' limited distribution area, slow growth rate and the uncertainties and variability in stock dynamics.

REFERENCES

Beverton, R. J. H. and Holt, S. J. 1966. Manual of methods for fish stock assessment. Part 2. Tables of yeld functions. FAO Fish. Tech. Pap. 38 (Rev. 1): 68 p.

Bllaca, M., Kolaneci, V. *2023.* Catch structure and CPUE of the main fish species caught in Ohrid Lake. In V. Balkan Agricultural Congress, 20-23 September, 2023, Edirne, Turkey.

Crawford, R., 1993. World record game fishes 1993. The International Game Fish Association, Pompano Beach, Florida.

Crivelli, A.J. 2006. *Salmo letnica*. *The IUCN Red List of Threatened Species* 2006: https://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T19858A9039230.en. (accessed on July 2024).

Gayanilo, F.C.Jr., Sparre, P., Pauly, D. (2005) FAO-ICLARM Stock Assessment Tools II (FiSAT II). Revised version. User's guide. FAO Computerized Information Series (Fisheries). 8, Revised version. Rome, FAO. 168 p.

INSTAT (Institute of Statistics, Albania). 2023. Fishery statistics for the year 2022. https://www.instat.gov.al/media/11801/njoftim-per-media-statistikat-e-peshkimit-2022.pdf (accessed on June, 2024)

Kostoski, G., Albrecht, C., Trajanovski, S., and Wilke, T. 2010. A freshwater biodiversity hotspot under pressure – assessing threats and identifying conservation needs for ancient Lake Ohrid, Biogeosciences, 7, 3999–4015.

Kottelat, M., and J. Freyhof. 2007. Handbook of European freshwater fishes. Publications Kottelat, Cornol, Switzerland.

MoARD (Ministry of Agriculture and Rural Development, Albania). Order No. 149, dated 31.3.2022 for the approval of the rregulations for the implementation of the Law No. 64, dated 31.5.2012 on "Fishery" (in Albanian).

Pauly, D. 1980. On the interrelationships between natural mortality, growth parameters, and mean environmental temperature in 175 fish stocks. J. Cons. Int. Explor. Mer., 39:175–192.

Pauly, D., 1984. Length-converted catch curves: a powerful tool for fisheries research in the tropics (Part II). ICLARM Fishbyte, 2(1): 17-19.

Pauly, D., 1990. Length-converted catch curves and the seasonal growth of fishes. ICLARM Fishbyte, 8(3): 33-38.

Schöffmann, J., Marić, S. (2023). Salmonid Fish Species: Opportunities for Sustainable Use under Multiple Pressures and Current Climatic Change. In: Simić, V., Simić, S., Pešić, V. (eds) Ecological Sustainability of Fish Resources of Inland Waters of the Western Balkans. Fish & Fisheries Series, vol 43.

Sparre, P., S. C. Venema, 1992. Introduction to tropical fish stock assessment, Part 1—manual. FAO Fish. Tech. Pap. 306/1 Rev. 1: 376 p.

Spirkovski Z, Ilik-Boeva D, Talevski T, Trajcevski B, Palluqi A, Flloko A, Miraku T, Kapedani E, Pietrock M, Ritterbusch D. 2017. Conservation and Sustainable Use of Biodiversity at Lakes Prespa, Ohrid and Shkodra/Skadar. Fish and Fisheries, Lake Ohrid. Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH.: 99 p.

Spirkovski, Z., Talevski, T. 2002. Investigations of Lake Ohrid fishes. Lake Ohrid Conservation Conference, Ohrid, Macedonia: 34-54.

HEALTH EFFECTS AND USES OF CHERRY LAUREL: A REVIEW

Tuğba ER[1]

[1]*Giresun Hazelnut Research Institute, Department of Food Technology, Türkiye*

Corresponding author e-mail: tugbaer2003@hotmail.com

ABSTRACT

Cherry laurel is an important fruit species that grows naturally in the Black Sea region of Turkiye. It attracts attention for its nutritional value and diverse uses, offering numerous health benefits due to its high antioxidant capacity and rich phenolic compounds. As a healthy food source, it is rich in vitamins, minerals, and dietary fibers and is widely utilized in traditional medicine. Its blood sugar-lowering, anti-inflammatory, and antioxidant properties make it an effective option for treating various health problems. Cherry laurel is used in products such as jam, marmalade, vinegar, liqueur, and compote, and is also valued in the landscaping and cosmetics sectors. The oil extracted from its seeds is used in the cosmetic industry. Studies on the bioactive compounds and positive health effects of cherry laurel indicate that this fruit is an attractive option for the functional foods and food supplements industries. In summary, increasing the recognition of cherry laurel and expanding its usage areas will both contribute to the fruit's genetic diversity in our country and provide significant benefits for public health. More research and promotional activities are required to fully evaluate the potential of cherry laurel.

Keywords: Taflan, traditional medicine, diabetes, *Prunus laurocerasus* L.

INTRODUCTION

Turkiye is one of the world's important fruit gene centers due to its geographical location and ecological conditions. Consequently, the country is the homeland of many fruit species and holds a significant place in fruit-growing culture. The Black Sea region of Türkiye, rich in natural resources and plant diversity, is particularly notable for its favorable ecological conditions. Among the various fruit types found in this region, cherry laurel stands out for its contribution to the fruit genetic diversity.

Cherry laurel (*Prunus laurocerasus L.*), belonging to the Rosaceae family, thrives in humid and temperate climates, preferring slightly acidic soils rich in organic matter. It grows naturally in the Black Sea region (*Laurocerasus officinalis Roem.*, syn: *Prunus laurocerasus L.*) and serves both as a fruit-bearing and ornamental plant. This evergreen tree or shrub, which retains its leaves year-round, can reach heights of 5-15 meters. The leaves are oval or elliptical, dark green, shiny, and have a hard structure with slightly toothed edges. In spring, the cherry laurel blooms with fragrant white or cream-colored flowers arranged in clusters (Table 1).

Table 1. Cherry laurel blooms (Damla ÇİL, 2023)

The fruit of the cherry laurel, approximately 6-25 mm in length, undergoes color changes as it ripens from green to red, purple, yellow, white, and finally dark, almost black, depending on the variety (Table 2). Fruit ripening begins in June and can extend until the end of August, depending on the genotype. The taste of the fruits ranges from sweet to astringent and bitter (Er, 2013).

Table 2. The fruit of the cherry laurel (Tuğba ER, 2024)

In Turkiye, cherry laurel is known by various names including taflan, Georgian cherry, laz grape, laz cherry, tanal, and tçkoo, with "taflan" being the most common (Anonymous, 2024). Recognizing the nutritional importance of cherry laurel, numerous studies have been conducted to cultivate and disseminate it (Karadeniz and Kalkisim, 1996; Sulusoglu, 2011; İslam and Deligöz, 2012).

The aim of this review is to enhance the recognition of cherry laurel fruit, which contributes significantly to the fruit genetic diversity of Turkiye. Despite its growth in many countries, its usage has not developed beyond local consumption or production. This review seeks to highlight its biochemical contents, areas of use, and health benefits.

BIOCHEMICAL CONTENT OF CHERRY LAUREL

The nutritional composition of cherry laurel includes a variety of macro and micronutrients that enhance its health value. This fruit contains high amounts of water, protein, and carbohydrates. It is also rich in pectin, an important dietary fiber. Additionally, cherry laurel is abundant in phenolic compounds (including flavonoids such as anthocyanin, flavonols, tannin, and lignin), vitamins (A, C, D), and minerals (K, Mg, Ca, P) (Karahalil et al., 2011; Kalyoncu et al., 2013). Studies on the nutritional content of cherry laurel have revealed its high antioxidant capacity, attributed to the rich phenolic compounds it contains (Ayaz, 2001; Var and Ayaz, 2004; Yazıcı et al., 2011; Ozturk et al., 2019; Celik et al., 2020).

The nutritional composition of cherry laurel fruit is influenced by several factors, including the place where it is grown, soil characteristics, season, and the maturity of the fruit. There are also numerous species of cherry laurel, leading to considerable variability in fruit content. For this reason, nutritional content information was taken from the National Food Composition database and is presented in Table 1.

Table 1. Nutrient Content of Cherry Laurel Fruit (100g)

Component	**Unit**	**Average**	**Component**	**Unit**	**Average**
Energy	Kcal/kJ	75/314	P	mg	21
Moisture	g	79,85	Ca	mg	45
Ash	g	0,68	Mg	mg	24
Protein	g	1,40	K	mg	166
Nitrogen	g	0,22	Na	mg	3
Oil, Total	g	0,54	Zn	mg	0,26
Carbohydrate	g	14,72	Vitamin C	mg	2,3
Fiber, total diet	g	2,81	L-ascorbik acid	mg	2,3
Fiber, water soluble	g	0,49	Riboflavin	mg	0,028
Fiber, water insoluble	g	2,32	Niacin	mg	0,277
Glucose	g	5,09	Vitamin B-6, total	mg	0,066
Fructose	g	6,36	Vitamin A	RE	2
Salt	mg	7	Beta-carotene	µg	19
Fe	mg	0,55	Lutein	µg	29

*Reference: Türkomp, 2024.

The most abundant mineral in cherry laurel is potassium. In addition to potassium, other important minerals found in cherry laurel include calcium, magnesium, and phosphorus. The fruit also contains natural sugars, primarily fructose and glucose, which vary depending on the fruit's development and ripening process (Ayaz, 2001). The sweet and slightly sour taste of cherry laurel is due to these sugars combined with the fruit's natural acidity.

Cherry laurel's high antioxidant capacity is attributed to its rich phenolic compounds, particularly vanillic acid, known for its anti-inflammatory effects (Ayaz, 2001; Var and Ayaz, 2004; Yazıcı et al., 2011; Ozturk et al., 2019; Celik et al., 2020). The fruit's nutritional composition makes it a healthy snack and a nutritious addition to the diet. With its high vitamin and mineral content, strong antioxidant properties, and low-fat content, cherry laurel can be consumed as part of a balanced diet.

USES OF CHERRY LAUREL

Cherry laurel has a range of uses in both traditional and modern applications. The fruit, with its slightly bitter taste, is consumed fresh and is used in making jam and marmalade, enhancing these products with its rich aroma and taste. It is also added to compotes to impart flavor and scent.

In the Black Sea region, cherry laurel is grown both naturally and partly through cultivation. Locally, it is consumed fresh and used in forms such as jam, molasses, and salting. Liqueur is also made from its fruits, and in recent years, the fruit has been consumed dried. In the Eastern Black Sea Region, cherry laurel leaves are brewed as tea, serving as a cough suppressant and antispasmodic for nervous disorders (FAE, 2024). The fruits are also considered diet-friendly due to their easy digestibility and ability to induce a feeling of fullness (Çalışır et al., 2002).

Studies on the use of cherry laurel in food products have generally yielded positive results. However, there has not been a significant increase in its use within the food industry. Increased research on its industrial applications is likely to boost its commercial value (Vahapoğlu et al., 2018).

Beyond its culinary uses, cherry laurel is valued as an ornamental plant in landscaping due to its evergreen nature. Its glossy leaves are used in making wreaths, floral arrangements, fish displays, and even as fodder for small ruminants. Additionally, the leaves are used to obtain aqua laurocerasi or laurocerasin for the pharmaceutical industry (Anonymous, 2024). The oil extracted from the seeds is utilized in the cosmetic industry. Consequently, cherry laurel has also become an attractive fruit for the functional foods and food supplements industries.

EFFECTS ON HEALTH

Cherry laurel is traditionally used in Türkiye to treat various health problems. The fruit and different parts of the plant have been utilized for their analgesic, antispasmodic, sedative, and blood sugar-lowering effects (Kolaylı et al., 2003). In the Eastern Black Sea Region, it is particularly valued for its ability to lower blood sugar levels. Additionally, it has been noted for its anti-inflammatory effects without causing gastric lesions (Erdemoglu et al., 2003).

Traditionally, cherry laurel fruits and seeds are recommended for treating digestive and respiratory diseases, bronchitis, eczema, and hemorrhoids (Çolak et al., 2005; Kolaylı et al., 2003). The high antioxidant content of the fruit helps reduce oxidative stress caused by free radicals in the body, thereby preventing cell damage. Polyphenols and flavonoids in the fruit may also protect heart health and prevent cardiovascular diseases.

Cherry laurel contains various glycosides, including cyanogenic glycosides such as amygdalin and prunasin. These compounds can release cyanide when hydrolyzed, offering potential health benefits but requiring caution due to toxicity risks. Like the fruit, cherry laurel leaves are used in traditional Turkish medicine to treat asthma, cough, and dyspepsia (Yeşilada et al., 1999).

Interest in cherry laurel has increased in recent years due to its high antioxidant and bioactive compound content. Studies suggest it may be effective in treating and preventing various diseases, including diabetes, cancer, cardiovascular, chronic, and neurodegenerative diseases (Karataş and Uçar, 2018).

CONCLUSION

Cherry laurel, abundant in our country, particularly in the Black Sea Region, stands out not only for its high water, protein, and carbohydrate content but also for its richness in phenolic compounds, vitamins, and minerals. This nutritional profile grants it a robust antioxidant capacity, making it a valuable asset for health. Traditional medicine has long recognized its analgesic, antispasmodic, and sedative properties, alongside documented benefits in lowering blood sugar and reducing inflammation.

Beyond its fresh consumption, cherry laurel is processed into diverse culinary delights such as jams, marmalades, vinegars, liqueurs, and dried fruits. It also finds use in landscaping as an ornamental plant. Recent biochemical research underscores its potential to enhance both commercial and industrial value. Encouraging findings suggest it could play a pivotal role in preventing and treating chronic ailments, including diabetes, cancer, cardiovascular diseases, and neurological disorders.

Increasing awareness and expanding applications of cherry laurel promise to elevate its economic significance and public health impact. Future strides in scientific inquiry and industrial innovation are poised to unveil its full potential, enabling broader consumption and furthering its beneficial contributions to society.

REFERENCES

Ayaz, F. A., 2001. Changes in phenolic acids of cherry laurel (*Laurocerasus officinalis* Oxygemmis) fruit during maturation. Acta Biologica Cracoviensia. Series Botanica, 43, 23-46.

Celik, O. F., Demirkol, M., Durmus, Y., & Tarakci, Z., 2020. Effects of drying method on the phenolics content and antioxidant activities of cherry laurel (*Prunus laurocerasus* L.). Journal of Food Measurement and Characterization, 14(1), 48-54.

Colak, A., Özen, A., Dincer, B., Güner, S., Ayaz, F.A., 2005. Diphenolases from two cultivars of cherry laurel (*Laurocerasus officinalis* Roem.) fruits at an early stage of maturation. Food Chem. 90 (4), 801–807.

Çalışır S., Aydın C., 2002. Some physico-mechanic proper-ties of cherry laurel (*Prunus lauracerasus* L.) fruits. Journal of Food Engineering 2004; 65:145-150. ve İslam A. ‘Kiraz’cherry laurel (*Prunus laurocerasus*). Journal of Crop and Horticultural Science 2002; 30:301-302.

Er, T.,2013. Karayemiş (*Prunus laurocerasus* L.). Giresun Fındık Araştırma Enstitüsü Müdürlüğü Er, 2013, Yayın No:26.

Erdemoglu, N., Küpeli, E., Yes¸ İlada, E., 2003. Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine. J. Ethnopharmacol. 89 (1), 123–129.

FAE, 2024. Fındık Araştırma Enstitüsü Karayemiş Yetiştiriciliği, https://arastirma.tarimorman.gov.tr/findik/Sayfalar/ Erişim Tarihi: 08.06.2024

Islam, A., & Deligöz, H., 2012. Selection on cherry laurel (*Laurocerasus officinalis* L.) in Ordu region. Akademik Ziraat Dergisi, 1(1), 37-44.

Kalyoncu, İ.H., Ersoy, N., Elidemir, A.Y., Dolek, C. 2013. Mineral and some physico-chemical composition of 'Karayemiş' (Prunus laurocerasus L.) fruits grown in Northeast Turkey, World Academy of Science, Engineering and Technology, 7(6), 11-14.

Karadeniz, T., & Kalkisim, O., 1996. A study on selection of promising native cherry laurel (*Prunus laurocerasus* L.) genotypes from Akcaabat, Turkey. Yuzuncu Yıl University Journal of Agricultural Sciences, 6, 147-153.

Karahalil FY, Şahin H., 2011. Phenolic composition and antioxidant capacity of Cherry laurel (*Laurocerasus officinalis* Roem.) sampled from Trabzon region, Turkey. African Journal of Biotech-nology 2011; 10:16293-16299.

Karataş, E. ve Uçar, A.,2018. Karayemiş'in Sağlık Üzerine Etkisi. Sağlık Bilimleri Dergisi (Journal of Health Sciences) 2018 ; 27 (1)

Kolayli, S., Kücük, M., Duran, C., Candan, F., Dincer, B., 2003. Chemical and antioxidant properties of *Laurocerasus officinalis* Roem. (cherry laurel) fruit grown in the Black Sea region. J. Agric. Food Chem. 51 (25), 7489–7494.

Ozturk, B., Karakaya, O., Yıldız, K., & Saracoglu, O., 2019. Effects of Aloe vera gel and MAP on bioactive compounds and quality attributes of cherry laurel fruit during cold storage. Scientia Horticulturae, 249, 31-37.

Sulusoglu, M., 2011. The cherry laurel (*Prunus laurocerasus* L.) tree selection. African Journal of Agricultural Research, 6(15), 3574-3582.

Türkomp, 2024. Ulusal Gıda Kompozisyon Veri Tabanı (Türkomp). Erişim yeri: https://turkomp.tarimorman.gov.tr/food-570 Erişim tarihi: 16.06.2024

Vahapoğlu, B., Altan, E.N., Gülseren, İ. (2018). Karayemiş meyvesinin biyoaktif özellikleri ve fonksiyonel gıdalarda kullanım potansiyeli. GIDA (2018) 43 (5): 751-764 doi: 10.15237/gida.GD18055

Var, M., & Ayaz, F. A., 2004. Changes in sugar composition in cherry laurel (cv oxygemmis) fruit during development and ripening. Pakistan Journal of Botany, 36(2), 389-394.

Yazıcı, K., Çakır, B., Kazaz, S. 2011. An important genetic resource for Turkey, Cherry laurel (*Laurocerasus officinalis* Roemer), Acta Horticulturae, 890, 261- 265.

Yeşilada E., Sezik, E., Honda, G., Takaishi, Y., Takeda, Y., and Tanaka, T.,1999. Traditional medicine in Turkey IX:: Folk medicine in north-west Anatolia. Journal of Ethnopharmacology 1999; 64:195-210.

INTERACTION OF CHRYSIN WITH MOLECULAR TARGETS OF PARKINSON'S DISEASE: AN *IN SILICO* APPROACH

Feyza BURUL[1], Selma SEZEN[1], Mehmet KARADAYI[2], Yusuf GÜLŞAHİN[2], Ufuk OKKAY[3], Medine GÜLLÜCE[2]

[1] Department of Medical Pharmacology, Faculty of Medicine, Agri Ibrahim Cecen University, Agri, Turkey
[2] Department of Biology, Ataturk University, Faculty of Science, Erzurum, Turkey
[3] Department of Medical Pharmacology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

Corresponding author e-mail: fburul@agri.edu.tr

ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease characterized by dopaminergic neuron loss in the substantia nigra. The incidence of the disease, which has symptoms such as bradykinesia, rigidity, and tremor, increases with age. Although the mechanism of the disease, which affects millions of people, is not fully understood, it is reported that protein aggregation due to α-synuclein misfolding, neuroinflammation, and many gene mutations, including LRRK2, contribute to the development of PD. Chrysin is a flavone compound found in high amounts in honey, propolis, and various plants, and its anti-inflammatory and neuroprotective effects have been shown in various studies. In the *in silico* study, the binding scores of neuroprotective chrysin with Parkinson's disease-specific (α-synuclein, LRRK2, MAO-B) and neuroinflammation-related (mTOR, MAPK, JNK3, iNOS, nNOS) proteins were recorded and the interaction levels were evaluated. The 3D models of the target proteins selected for molecular docking studies were obtained from the RCSB Protein Data Bank with the ID numbers α-synuclein (1XQ8), LRRK2 (5OPB), MAO-B (1GOS), mTOR (2FAP), MAPK (5UOJ), JNK3 (2O0U), iNOS (3HR4) and nNOS (5VV0). The 3D structures of the ligand tests were obtained from DrugBank and NCBI PubChem databases and their structural minimizations were performed with USCF Chimera ver. 1.17.1. After the receptors and ligands were prepared with AutoDockTools 1.5.7, binding scores were recorded. In the obtained findings, it was shown *in silico* that chrysin interacts with α-synuclein, LRRK2, MAO-B, mTOR, MAP JNK3, iNOS and nNOS receptor proteins. The *in silico* findings obtained for chrysin, which targets protein aggregation through the interaction with α-synuclein and LRRK2, neuroinflammation through the interaction with mTOR, MAPK, JNK3, iNOS and nNOS, and the dopaminergic system through the interaction with MAO-B, demonstrate the potential effect of the target compound in Parkinson's disease and shed light on new studies to be conducted for the treatment of the disease.

Keywords: Chrysin, Molecular Docking, Neuroinflammation, Parkinson’s Disease

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease worldwide, characterized by loss of dopaminergic neurons in the substantia nigra and misfolding of α-synuclein. The prevalence of the disease is expected to reach 12 million by 2040 with increasing age (Dorsey et al., 2018). Symptoms include bradykinesia, rigidity, tremor, postural instability, constipation, dementia, and sleep disorders, and current treatments for PD are only symptomatic. The pathogenesis of PD, which is associated with many mechanisms including oxidative stress, neuroinflammation and autophagy, has not been fully elucidated. In addition, the symptomatic nature of current PD treatment negatively affects the quality of life of patients. This situation increases the need for new treatment approaches for PD and causes researchers to focus on neuroprotective compounds (Jankovic et al., 2008; Cabreira et al., 2019; Angelopoulou et al., 2020).

Neuroinflammation, which aggravates neurodegeneration in the pathogenesis of PD, is induced by various factors. These factors include pathogens, prions, toxins, α-synuclein aggregates, and proinflammatory cytokines that cause activation of microglia expressed in the central nervous system (CNS) (Araújo et al., 2020). Activated microglia are closely associated with α-synuclein aggregation as well as an increase in proinflammatory cytokines. This results in an exacerbation of neuroinflammation (Li et al., 2021). α-synuclein, which has an important role in neuroinflammation, is a water-soluble protein in its normal form. α-synuclein aggregates are formed as a result of misfolding of α-synuclein. α-synuclein aggregates, which are used as biomarkers in the diagnosis of PD, have also been detected at high levels in the cerebsrospinal fluid (CSF) of Parkinson's patients. These aggregates induce neuroinflammation through various mechanisms, thereby exacerbating neurodegeneration (Liu et al., 2022). Leucine-rich repeat kinase 2 (LRRK2), which is associated with α-synuclein overexpression and increased neuroinflammation, plays a role in various pathways and cellular signals, including autophagy, in the pathogenesis of PD. It is known that LRRK2 mutations constitute the most common causes of familial and sporadic PD, and LRRK2 inhibition is used as a treatment strategy (Rosenbusch et al., 2016; Di Maio et al., 2018; Liu et al., 2022). In addition to LRRK2, inhibition of the mechanistic target of rapamycin (mTOR), which is associated with α-synuclein aggregation, has also been reported to have neuroprotective effects. mTOR, which regulates synaptic plasticity, neuronal development and survival, has also been closely associated with microglia activation, which aggravates neuroinflammation with protein aggregation (Lan et al., 2017; Dhapola et al., 2021). Many stress factors that activate microglia, especially proinflammatory cytokines, also induce mitogen-activated protein kinase (MAPK). MAPK, which regulates many physiological processes such as cellular stress response and cell death and intracellular signaling, also mediates pathways related to neuroinflammation, apoptosis and oxidative stress (Jayaraj et al., 2019; Ratih et al., 2023). However, it is known that c-Jun N-terminal kinase-3 (JNK3), which is also in the MAPK family, is intensely expressed in the CNS and plays a role in the pathogenesis of PD. In this context, MAPK and JNK3 are considered an important therapeutic target for PD in alleviating neuroinflammation (Bohush et al., 2018).

Oxidative stress, which plays an intricate role with neuroinflammation in PD, is known to be one of the important factors leading to neurodegeneration. Inducible nitric oxide synthase (iNOS), which increases oxidative stress levels, causes dopaminergic neuron death together with increased nitric oxide (NO) levels. In an *in vitro* PD model induced with 6-hydroxidopamine (6-OHDA), chrysin has been shown to reduce oxidative stress caused by iNOS (Zhang et al., 2015). In addition to iNOS, neuronal nitric oxide synthase (nNOS) also has an effect on increasing NO levels. NO demonstrates its neurodegenerative effect by altering axo-dendritic function, disrupting synaptic signaling, vesicular traffic, and dopamine homeostasis (Stykel et al., 2022). The decrease in dopamine activity along with the disruption of homeostasis worsens PD symptoms and increases degeneration. Monoamine oxidase-B (MAO-B), which metabolizes dopamine in striatal glial cells, also catalyzes the oxidation of various neurotransmitters. The decreased dopamine levels in PD are increased through MAO-B inhibition, and MAO-B inhibitors are used for PD treatment (Regensburger et al., 2023). In addition to dopaminergic activity, it has been reported that MAO-B is also closely associated with PD-specific α-synuclein and that the increase in MAO-B expression due to age triggers neurodegeneration through free radical damage (Kang et al., 2018). In this context, alleviating neuroinflammation and oxidative stress that worsen PD symptoms, increasing dopaminergic activity, and autophagy regulation are strategic approaches in PD treatment. However, determining the bioactivities and mechanisms of action of components obtained from natural products with low toxicity will enable the synthesis of these components or their new derivatives with increased activity and their use in the prophylaxis of neurodegenerative diseases.

Chrysin (5,7-dihydroxyflavone or 5,7-dihydroxy-2-phenyl-4H-chromen-4-one) is a bioactive flavonoid found in many plants such as *Oroxylum indicum*, *Passiflora incarnata* and *Passiflora caerulea*, mushrooms, honey and propolis (Figure 1). Chrysin, which has been shown to cross the blood-brain barrier, has been reported to have anticancer, antidiabetic, anti-inflammatory, hepatoprotective, cardioprotective, antioxidant, antiepileptic and neuroprotective effects. Chrysin is known to exhibit its neuroprotective effects by reducing various proinflammatory cytokine levels and oxidative stress (Naz et al., 2019; Angelopoulou et al., 2020).

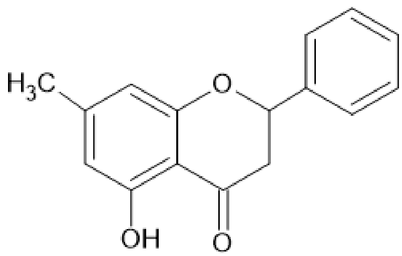


Figure 1. The structure of chrysin (Created by ACD/ChemSketch)

When the data in the literature is evaluated, chrysin’s neuroprotective effects has been shown in limited number of studies in PD, however the exact mechanism has not yet been clearly elucidated. In this study, for the first time, the binding scores of chrysin with PD-specific (α-synuclein, LRRK2, MAO-B) and neuroinflammation-related receptors (mTOR, MAPK, JNK3, iNOS, nNOS) that play a role in PD pathogenesis were determined using the molecular docking analysis method and the aim was to elucidate the mechanism of chrysin in PD.

MATERIAL AND METHOD

The 3D structure of chrysin used as a ligand for molecular docking analyses was downloaded from the online Drugbank database with the code DB15581. (DrugBank, https://go.drugbank.com/ accessed on 25 April 2024). Minimization of the ligand chrysin was performed with USCF Chimera ver. Implemented with 1.17.1 software and prepared for molecular docking analysis with the AutoDockTools-1.5.7 program. Experimentally elucidated structures of target receptors known to be associated with PD were obtained from the RCSB Protein Data Bank with the ID numbers α-synuclein (1XQ8), LRRK2 (5OPB), MAO-B (1GOS), mTOR (2FAP), MAPK (5UOJ), JNK3 (2O0U), iNOS (3HR4) and nNOS (5VV0).

(PDB, www.rcsb.org accessed on 25 April 2024). After preparing the receptors with AutoDockTools-1.5.7, the binding score of each receptor with chrysin was recorded (Garcia et al., 2023).

RESULTS AND DISCUSSION

Chrysin is a polyphenolic compound with many activities such as antioxidant, anti-inflammatory, neuroprotective and anticancer. It has been reported that chrysin plays a neuroprotective role in neurodegenerative diseases such as Alzheimer's disease (AD), epilepsy and PD, and the mechanisms through which it performs these effects are still a subject of research (Mishra et al., 2021). Findings obtained from molecular docking analyses showed that chrysin has significant binding affinity with PD-specific (α-synuclein, LRRK2, MAO-B) and neuroinflammation-related receptors (mTOR, MAPK, JNK3, iNOS, nNOS) that play a role in PD pathogenesis. Chrysin binding scores and interactions are visualized in Figure 2-9 and summarized in Table 1.

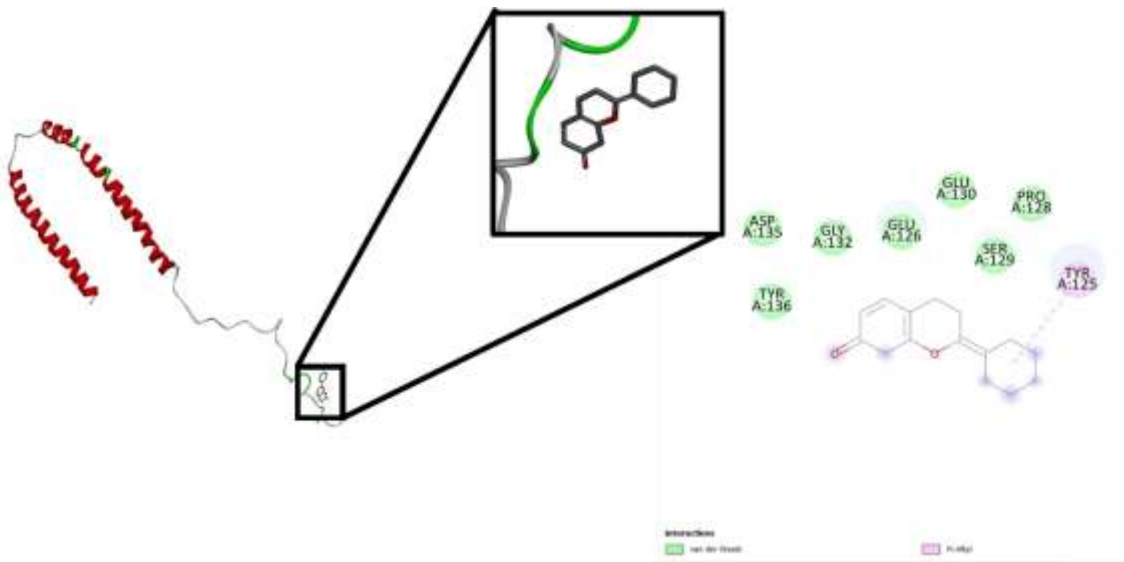


Figure 2. 3D and 2D interaction diagrams of chrysin-α-synuclein complex (Score: -6.4 kcal/mol)

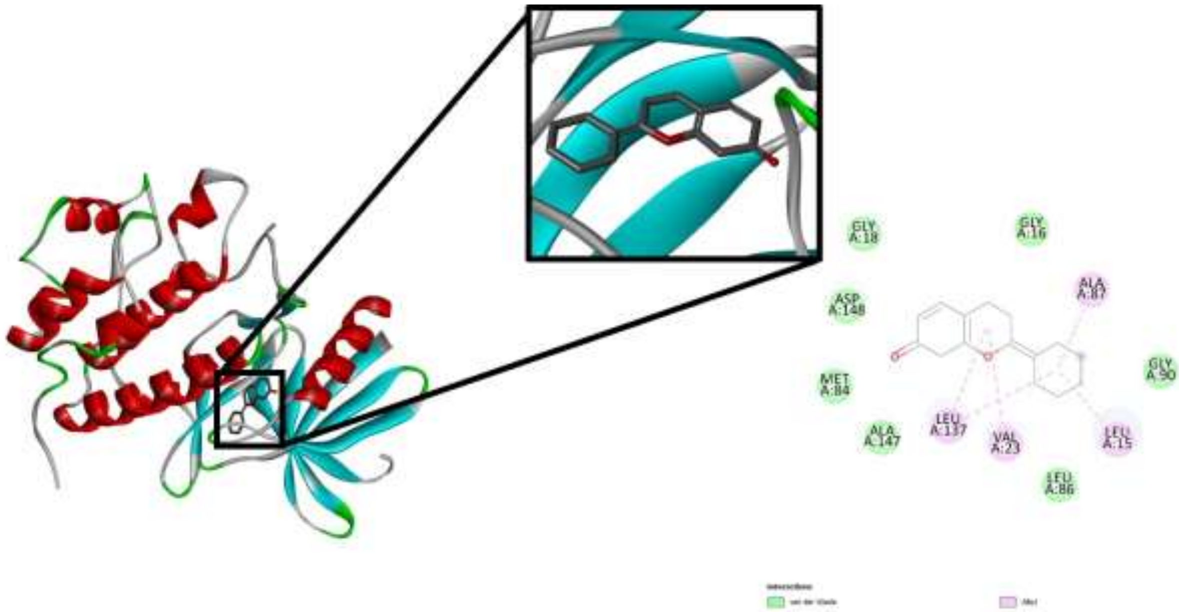


Figure 3. 3D and 2D interaction diagrams of the chrysin-LRRK2 complex (Score: -9.3 kcal/mol)

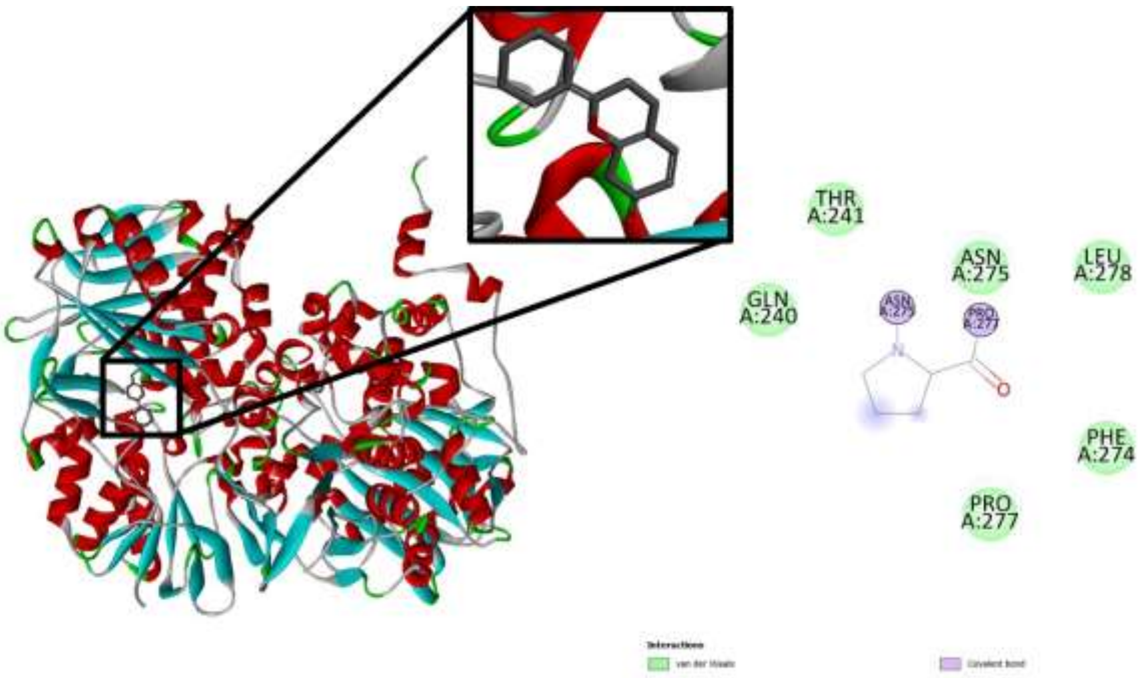


Figure 4. 3D and 2D interaction diagrams of the chrysin-MAO-B complex (Score: -9.1 kcal/mol)

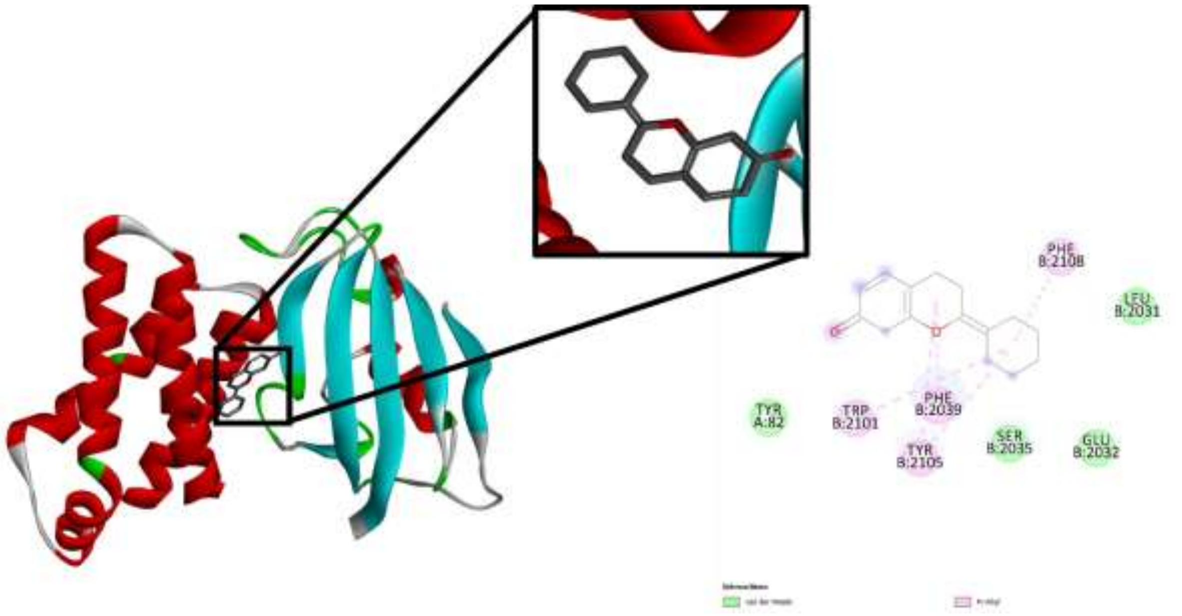


Figure 5. 3D and 2D interaction diagrams of the chrysin-mTOR complex (Score: -8.6 kcal/mol)

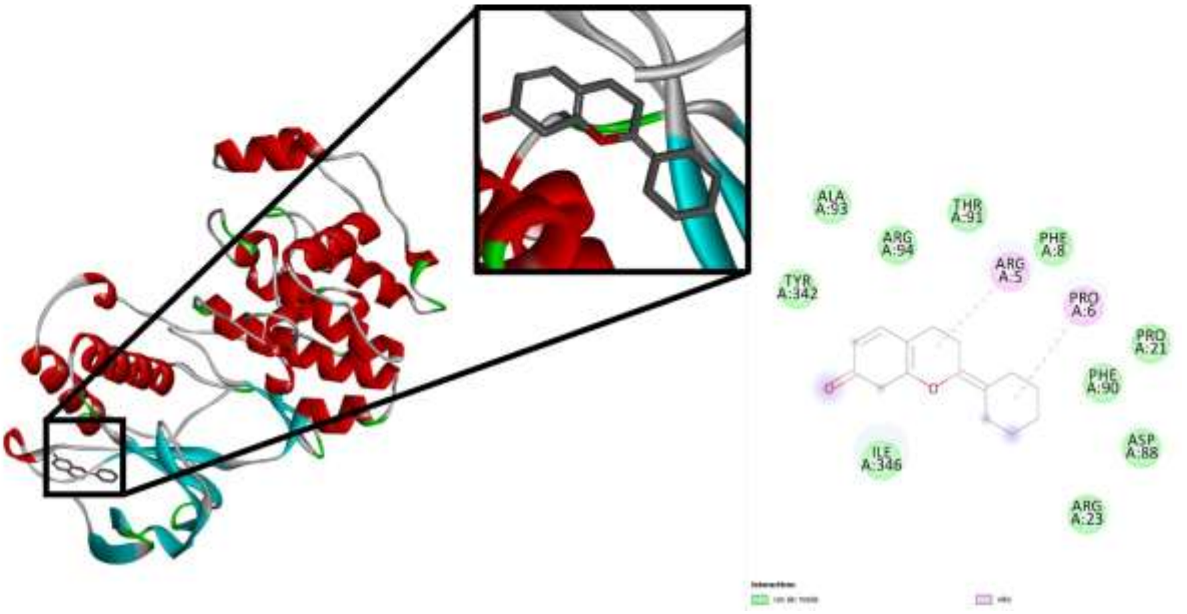


Figure 6. 3D and 2D interaction diagrams of the chrysin-MAPK complex (Score: -7.5 kcal/mol)

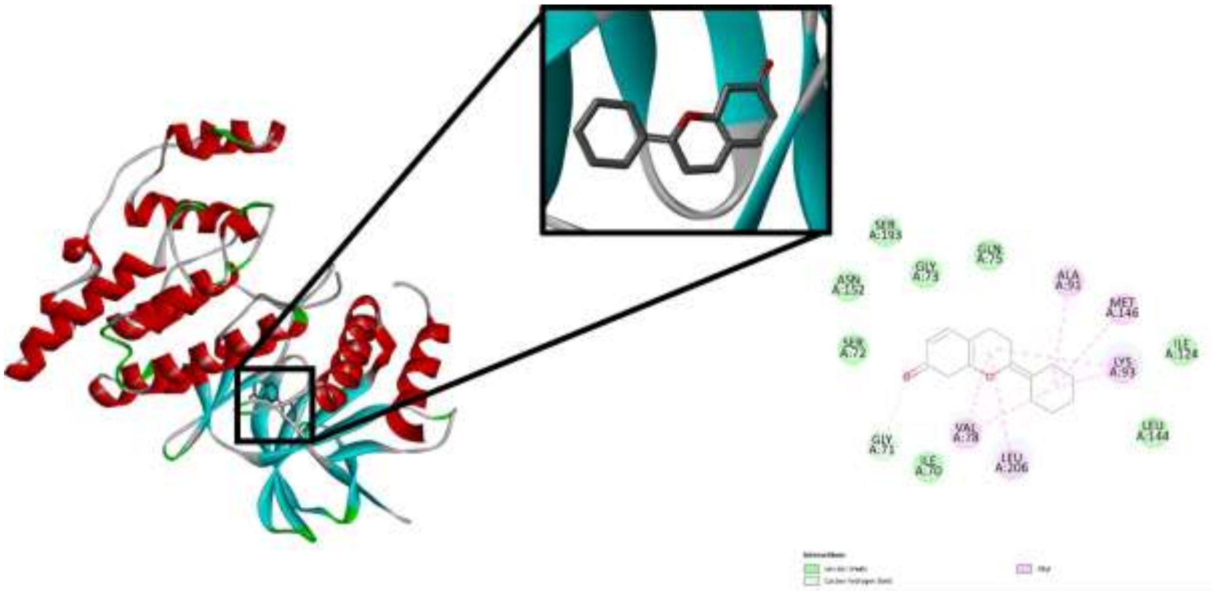


Figure 7. 3D and 2D interaction diagrams of the chrysin-JNK3 complex (Score: -8.4 kcal/mol)

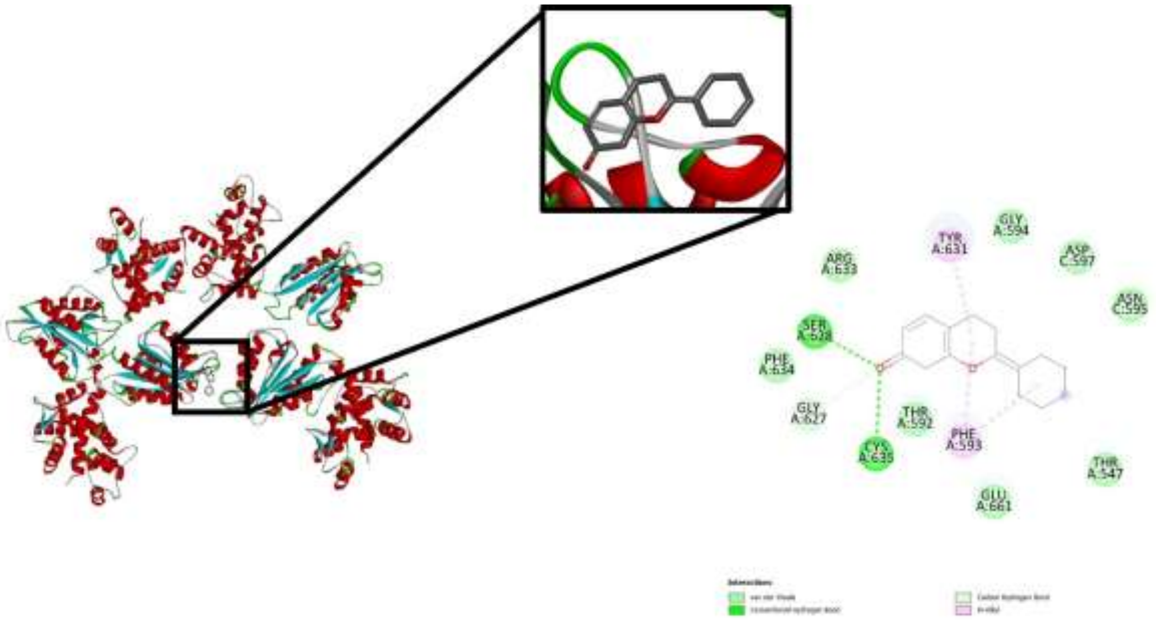


Figure 8. 3D and 2D interaction diagrams of the chrysin-iNOS complex (Score: -9.9 kcal/mol)

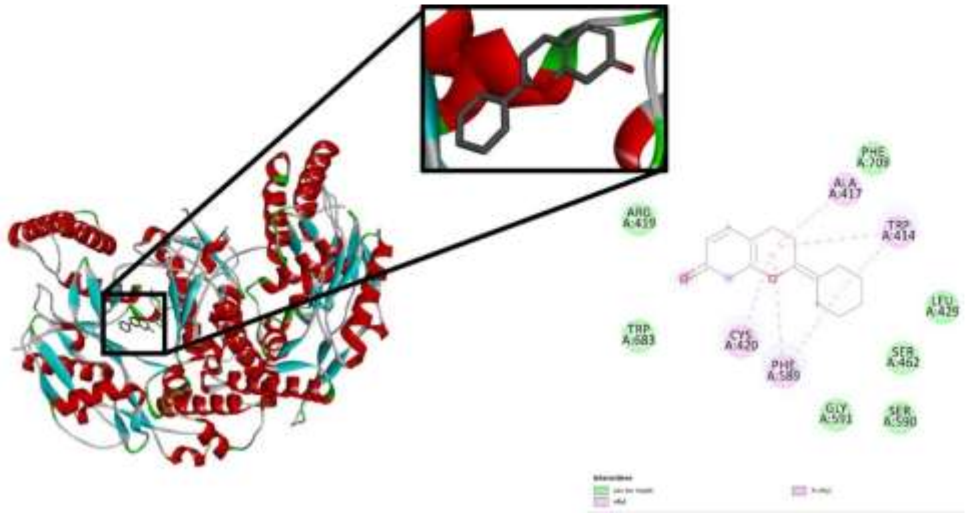


Figure 9. 3D and 2D interaction diagrams of the chrysin-nNOS complex (Score: -9.6 kcal/mol)

Table 1. Binding scores of chrysin and PD-related receptors

Receptor	**PDB ID**	**Interacting Residues**	**Docking Score (kcal/mol)**
α-synuclein	1XQ8	TYR A:125	-6.4
LRRK2	5OPB	ALA A:87 LEU A:15 LEU A:137 VAL A:23	-9.3
mTOR	2FAP	PHE B:2108 PHE B:2039 TYR B:2105 TRP B:2105	-8.6
MAPK	5UOJ	ARG A:5 PRO A:6	-7.5
JNK3	2O0U	ALA A:91 MET A:146 LEU A:206 LYS A:93 VAL A:78	-8.4
iNOS	3HR4	PHE A:593 TYR A:631	-9.9
nNOS	5VV0	ALA A:417 CYS A:420 PHE A:589 TRP A:414	-9.6
MAO-B	1GOS	ASN A:275 PRO A:277	-9.1

As a result of the findings obtained from *in silico* analyses, we determined the binding affinity of chrysin with PD-specific α-synuclein (-6.4 kcal/mol). It has been shown that chrysin reduces α-synuclein aggregation and dopamine degeneration in a 6-OHDA-induced PD model (Muhammed et al., 2022). It has been stated that chrysin, which has been shown to protect dopaminergic activity by reducing dopamine depletion in alleviating degeneration, can provide a significant improvement in dopamine levels in the hippocampus and prefrontal cortex regions of the brain (Mishra et al., 2021). Our *in silico* findings also showed that chrysin exhibits a strong interaction with MAO-B, which plays a role in dopamine homeostasis. At the same time, MAO-B inhibitors, which have been shown to delay the formation of α-synuclein aggregation, have been evaluated as an effective treatment strategy in preserving dopaminergic activity (Tan et al., 2022). In addition to dopaminergic activity, LRRK2, which is characterized by PD, leads to oxidative damage and disruptions in the autophagy pathway. It has been determined that LRRK2 inhibition alleviates neuroinflammation together with microglial activation, and LRRK2 inhibition has gained importance in PD treatment (Ilieva et al., 2024). LRRK2 also bound to chrysin with a high binding score (-9.3 kcal/mol) in *in silico* analyses. This demonstrates the potential of chrysin to exhibit its neuroprotective effect through LRRK2 inhibition. mTOR, which is known to mediate microglia activation, also induces iNOS via inflammatory pathways (Dhapola et al., 2021). In a study, an *in vivo* PD model was induced with 6-OHDA and neuroprotective effect was obtained by inhibiting nNOS, which is known to increase NO levels, as well as iNOS (Tiwari et al., 2022). In *in silico* analyses, the high binding affinity of chrysin to mTOR (-8.6 kcal/mol), iNOS (-9.9 kcal/mol) and nNOS (-9.6 kcal/mol), which are associated with neuroinflammation, makes these receptors a target for chrysin's effects in PD. In addition, it has been reported that chrysin plays a neuroprotective role in the alleviation of neuroinflammation by modulating JNK expression and directly reducing the amount of proinflammatory cytokines (Mishra et al., 2021). The high binding affinity of chrysin with JNK3 (-8.4 kcal/mol) and its superfamily MAPK (-7.5 kcal/mol) reveals the potential effect of chrysin in the mechanism of response to cellular stress.

CONCLUSIONS

Our findings from *in silico* analyses have shown that chrysin has the potential to affect PD by interacting with α-synuclein, LRRK2, MAO-B, mTOR, MAPK, JNK3, iNOS and nNOS. However, extended preclinical studies are needed due to unpredictable physiological processes.

REFERENCES

Angelopoulou, E., Pyrgelis, E. S., & Piperi, C. (2020). Neuroprotective potential of chrysin in Parkinson's disease: Molecular mechanisms and clinical implications. Neurochemistry international, 132, 104612.

Araújo, B., Caridade-Silva, R., Soares-Guedes, C., Martins-Macedo, J., Gomes, E. D., Monteiro, S., & Teixeira, F. G. (2022). Neuroinflammation and Parkinson's Disease-From Neurodegeneration to Therapeutic Opportunities. Cells, 11(18), 2908.

Bohush, A., Niewiadomska, G., & Filipek, A. (2018). Role of Mitogen Activated Protein Kinase Signaling in Parkinson's Disease. International journal of molecular sciences, 19(10), 2973.

Cabreira, V., & Massano, J. (2019). Doença de Parkinson: Revisão Clínica e Atualização [Parkinson's Disease: Clinical Review and Update]. Acta medica portuguesa, *32*(10), 661–670.

Del Fabbro, L., Rossito Goes, A., Jesse, C. R., de Gomes, M. G., Cattelan Souza, L., Lobo Ladd, F. V., Lobo Ladd, A. A. B., Nunes Arantes, R. V., Reis Simionato, A., Oliveira,

M. S., Furian, A. F., & Boeira, S. P. (2019). Chrysin protects against behavioral, cognitive and neurochemical alterations in a 6-hydroxydopamine model of Parkinson's disease. Neuroscience letters, 706, 158–163.

Dhapola, R., Hota, S. S., Sarma, P., Bhattacharyya, A., Medhi, B., & Reddy, D. H. (2021). Recent advances in molecular pathways and therapeutic implications targeting neuroinflammation for Alzheimer's disease. Inflammopharmacology, 29(6), 1669–1681.

Di Maio, R., Hoffman, E. K., Rocha, E. M., Keeney, M. T., Sanders, L. H., De Miranda, B. R., Zharikov, A., Van Laar, A., Stepan, A. F., Lanz, T. A., Kofler, J. K., Burton, E. A., Alessi, D. R., Hastings, T. G., & Greenamyre, J. T. (2018). LRRK2 activation in idiopathic Parkinson's disease. Science translational medicine, 10(451), 5429.

Dorsey, E. R., & Bloem, B. R. (2018). The Parkinson Pandemic-A Call to Action. JAMA neurology, 75(1), 9–10.

Garcia, P. J. B., Huang, S. K., De Castro-Cruz, K. A., Leron, R. B., & Tsai, P. W. (2023). In Silico Neuroprotective Effects of Specific Rheum palmatum Metabolites on Parkinson's Disease Targets. International journal of molecular sciences, 24(18), 13929.

Guo, B., Zheng, C., Cai, W., Cheng, J., Wang, H., Li, H., Sun, Y., Cui, W., Wang, Y., Han, Y., Lee, S. M., & Zhang, Z. (2016). Multifunction of Chrysin in Parkinson's Model: Anti-Neuronal Apoptosis, Neuroprotection via Activation of MEF2D, and Inhibition of Monoamine Oxidase-B. Journal of agricultural and food chemistry, 64(26), 5324–5333.

Ilieva, N. M., Hoffman, E. K., Ghalib, M. A., Greenamyre, J. T., & De Miranda, B. R. (2024). LRRK2 kinase inhibition protects against Parkinson's disease-associated environmental toxicants. Neurobiology of disease, 196, 106522.

Jankovic J. (2008). Parkinson's disease: clinical features and diagnosis. Journal of neurology, neurosurgery, and psychiatry, 79(4), 368–376.

Jayaraj, R. L., Azimullah, S., Beiram, R., Jalal, F. Y., & Rosenberg, G. A. (2019). Neuroinflammation: friend and foe for ischemic stroke. Journal of neuroinflammation, 16(1), 142.

Kang, S. S., Ahn, E. H., Zhang, Z., Liu, X., Manfredsson, F. P., Sandoval, I. M., Dhakal, S., Iuvone, P. M., Cao, X., & Ye, K. (2018). α-Synuclein stimulation of monoamine oxidase-B and legumain protease mediates the pathology of Parkinson's disease. The EMBO journal, 37(12), e98878.

Lan, A. P., Chen, J., Zhao, Y., Chai, Z., & Hu, Y. (2017). mTOR Signaling in Parkinson's Disease. Neuromolecular medicine, 19(1), 1–10.

Li, Y., Xia, Y., Yin, S., Wan, F., Hu, J., Kou, L., Sun, Y., Wu, J., Zhou, Q., Huang, J., Xiong, N., & Wang, T. (2021). Targeting Microglial α-Synuclein/TLRs/NF-kappaB/NLRP3 Inflammasome Axis in Parkinson's Disease. Frontiers in immunology, 12, 719807.

Liu, T. W., Chen, C. M., & Chang, K. H. (2022). Biomarker of Neuroinflammation in Parkinson's Disease. International journal of molecular sciences, 23(8), 4148.

Mishra, A., Mishra, P. S., Bandopadhyay, R., Khurana, N., Angelopoulou, E., Paudel, Y. N., & Piperi, C. (2021). Neuroprotective Potential of Chrysin: Mechanistic Insights and Therapeutic Potential for Neurological Disorders. Molecules (Basel, Switzerland), 26(21), 6456.

Muhammad, F., Liu, Y., Wang, N., Zhao, L., Zhou, Y., Yang, H., & Li, H. (2022). Anti-α-synuclein Toxicity and Anti-neurodegenerative Role of Chrysin in Transgenic Caenorhabditis elegans Models of Parkinson's Disease. ACS chemical neuroscience, 13(4), 442–453.

Naz, S., Imran, M., Rauf, A., Orhan, I. E., Shariati, M. A., Iahtisham-Ul-Haq, IqraYasmin, Shahbaz, M., Qaisrani, T. B., Shah, Z. A., Plygun, S., & Heydari, M. (2019). Chrysin: Pharmacological and therapeutic properties. Life sciences, 235, 116797.

Ratih, K., Lee, Y. R., Chung, K. H., Song, D. H., Lee, K. J., Kim, D. H., & An, J. H. (2023). L-Theanine alleviates MPTP-induced Parkinson's disease by targeting Wnt/β-catenin signaling mediated by the MAPK signaling pathway. International journal of biological macromolecules, 226, 90–101.

Regensburger, M., Ip, C. W., Kohl, Z., Schrader, C., Urban, P. P., Kassubek, J., & Jost, W. H. (2023). Clinical benefit of MAO-B and COMT inhibition in Parkinson's disease: practical considerations. Journal of neural transmission (Vienna, Austria : 1996), 130(6), 847–861.

Rosenbusch, K. E., & Kortholt, A. (2016). Activation Mechanism of LRRK2 and Its Cellular Functions in Parkinson's Disease. Parkinson's disease, 2016, 7351985.

Stykel, M. G., & Ryan, S. D. (2022). Nitrosative stress in Parkinson's disease. NPJ Parkinson's disease, 8(1), 104.

Tan, Y. Y., Jenner, P., & Chen, S. D. (2022). Monoamine Oxidase-B Inhibitors for the Treatment of Parkinson's Disease: Past, Present, and Future. Journal of Parkinson's disease, 12(2), 477–493.

Tiwari, P. C., Chaudhary, M. J., Pal, R., & Nath, R. (2022). Effects of mangiferin and its combination with nNOS inhibitor 7-nitro-indazole (7-NI) in 6-hydroxydopamine (6-OHDA) lesioned Parkinson's disease rats. Fundamental & clinical pharmacology, 36(6), 944–955.

Zhang, Z., Li, G., Szeto, S. S. W., Chong, C. M., Quan, Q., Huang, C., Cui, W., Guo, B., Wang, Y., Han, Y., Michael Siu, K. W., Yuen Lee, S. M., & Chu, I. K. (2015). Examining the neuroprotective effects of protocatechuic acid and chrysin on in vitro and in vivo models of Parkinson disease. Free radical biology & medicine, 84, 331–343.

CURRENT STATUS AND POTENTIAL APPLICATION OF MICROALGAE PRODUCTION

Seyit Uguz[1], Arda Sozcu[2], Ercan Simsek[1], Erkan Yaslioglu[1]

[1] Department of Biosystem Engineering Bursa Uludag University, TURKEY
[2] Department of Animal Science Bursa Uludag University, TURKEY

seyit@uludag.edu.tr

ABSTRACT

Microalgae production has garnered significant attention across various industries due to its diverse applications and potential benefits. This study explores the current status and potential applications of microalgae production, focusing on its role in biodiesel production, cosmetics, thalassotherapy, bioremediation, and feed materials. The high lipid content of certain microalgae species makes them a promising source for sustainable biofuels, while their utilization in cosmetics and thalassotherapy underscores the value of microalgae-derived compounds in high-value products. Microalgae are also being explored for bioremediation purposes, particularly in treating textile wastewater, showcasing their versatility in environmental applications. Genetic engineering strategies are being developed to enhance biofuel production by increasing lipid accumulation in microalgae strains.

Keywords: Microalgae, feed, food, biofuel, treatment, waste

INTRODUCTION

Microalgae production has gained significant attention due to its diverse applications and potential benefits across various industries, including renewable energy, biopharmaceuticals, and nutraceuticals. The potential of microalgae production extends to biofuels, feed, food products, and other valuable compounds. Researchers have highlighted the rich energy content, rapid growth rate, cost-effective cultivation methods, and environmental benefits of microalgae, positioning them as a promising feedstock for biofuel generation. The current status of microalgae production is characterized by ongoing research and technological advancements aimed at maximizing the efficiency and scalability of microalgae cultivation. One prominent application of microalgae lies in biodiesel production, where the high lipid content of certain microalgae species presents a promising source for sustainable biofuels (Wu et al., 2012). Beyond biofuels, microalgae are being explored for bioremediation purposes, particularly in treating textile wastewater, showcasing the versatility of microalgae in environmental applications (Andrade & Andrade, 2018). The potential of microalgae extends to genetic engineering for enhanced biofuel production, with metabolic engineering strategies being developed to increase lipid accumulation in microalgae strains (Banerjee et al., 2016). Moreover, the biotechnological applications of microalgae have expanded to include the production of cosmetics, health products, fertilizers, feeds, and food, emphasizing the versatility of microalgae biomass in various industries (Ugya & Meguellati, 2022). Additionally, the utilization of microalgae in cosmetics and thalassotherapy has gained traction, highlighting the value of microalgae-derived compounds in high-value products (Mourelle et al., 2017).

The biotechnological potential of native microalgae from diverse ecosystems, such as the Peruvian Amazon, underscores the growing relevance of microalgae biotechnology in harnessing unique strains for specific applications (Cobos et al., 2020). Furthermore, the incorporation of microalgae into innovative food products and the extraction of valuable compounds like phytosterols for functional food and pharmaceutical applications highlight the growing interest in utilizing microalgae for health and nutrition purposes (Caporgno & Mathys, 2018; Luo et al., 2015). The development of low-cost and high-volume open microalgae biofuel production technologies signifies progress towards commercial-scale microalgae biofuel production, addressing the need for sustainable and renewable energy sources (Moheimani et al., 2013). Additionally, the genetic manipulation of microalgae for bioproducts and enhancing microalgae traits demonstrate the continuous advancements in biotechnology for optimizing microalgae-based applications (Vázquez-Villegas et al., 2018; Shin et al., 2016).

In conclusion, the current status and potential applications of microalgae production encompass a wide range of industries, from biofuels and cosmetics to bioremediation and feed products. The ongoing research and technological innovations in microalgae cultivation and genetic engineering are paving the way for a sustainable and versatile bio-based economy with microalgae at its forefront.

CURENT STATUS OF MICROALGAE PRODUCTION

Potential Applications

Food and feed material

Microalgae production is recognized for its potential as a sustainable and versatile source of food and feed materials, offering a wide array of nutritional and functional benefits.

Microalgae have historically been consumed by humans for their nutritional value and medicinal properties (Sathasivam et al., 2019).

Studies have demonstrated the feasibility of large-scale cultivation of microalgae for feed purposes, particularly in livestock and poultry production (Saadaoui et al., 2021). In the realm of food production, microalgae present a sustainable alternative as feed material for monogastric animals, providing a viable option to supplement or replace traditional feedstuffs like corn and soybean (Chaves et al., 2021). The nutritional composition of microalgae, which includes proteins, polyunsaturated fatty acids, antioxidants, and phytosterols, positions them as promising sources for food and feed applications (Sui & Vlaeminck, 2020). Additionally, the bioactive lipids found in microalgae have applications not only in aquaculture feed but also in the food and pharmaceutical industries, showcasing the versatility of microalgae-derived compounds (Luo et al., 2015).

Microalgae play a crucial role in aquaculture by serving as essential sources of polyunsaturated fatty acids for aquatic species (Soto-Sánchez et al., 2023). Their high nutritional value makes them suitable food sources for larval bivalves, shrimps, and fish, emphasizing their significance in aquaculture feed formulations (Ma, 2023). Furthermore, the industrial utilization of microalgae biomass includes the production of feeds, underscoring their importance in the food chain (Ugya & Meguellati, 2022). The nutritional composition and functional properties of microalgae proteins have been well-established, positioning them as a simple and effective protein source for food and feed applications (Hashim et al., 2023). Commercially, microalgae are already integrated into various food products, demonstrating their potential as versatile ingredients with nutritional benefits (Igual et al., 2021). The expanding industrial applications of microalgae encompass food, nutraceuticals, pharmaceuticals, animal feed, cosmetics, and biofertilizers, showcasing the diverse opportunities presented by these microorganisms (Udayan et al., 2021).

Microalgae production holds vast promise as food and feed material, providing sustainable and nutrient-rich alternatives for various industries. The nutritional value, bioactive compounds, and functional properties of microalgae make them valuable resources for addressing food scarcity, ensuring adequate nutrition, and promoting sustainable living practices.

Pharmaceutical and cosmetic industries

The pharmaceutical sector is increasingly exploring the therapeutic potential of microalgae-derived compounds, such as antioxidants, anti-inflammatory agents, antimicrobials, and anticancer agents, highlighting their pivotal role in biomedical applications (Khavari et al. 2021). These bioactive metabolites from microalgae hold significant promise for developing novel pharmaceutical products due to their diverse pharmacological activities (Kaur et al., 2023). In the cosmetic industry, microalgae have gained attention for their cosmetic and cosmeceutical applications, with compounds like astaxanthin being utilized as pigments in cosmetics and as active ingredients in skincare products (Villaró et al., 2021). The bioactive compounds present in microalgae, including pigments, vitamins, and minerals, offer a natural and sustainable source for formulating cosmetic products, catering to the increasing consumer demand for natural and eco-friendly alternatives (Dussably et al., 2022). Moreover, marine microalgae and cyanobacteria have been identified for their potential use in cosmetics and thalassotherapy, underscoring their significance in the cosmetic industry (Mourelle et al., 2017). The pharmaceutical and cosmetic industries are increasingly turning to microalgae as a valuable resource for developing high-value products. Microalgae's ability to produce a wide range of bioactive compounds, such as carotenoids, phytosterols, and fatty acids, positions them as versatile sources for pharmaceutical and cosmetic applications (Balasubramaniam et al., 2021). The bioactive metabolites synthesized by microalgae have demonstrated antioxidant, anti-inflammatory, and anti-microbial properties, making them attractive candidates for pharmaceutical formulations and cosmetic products (Morais et al., 2015). Furthermore, the sustainable production of microalgae biomass offers a cost-effective and environmentally friendly approach to sourcing bioactive compounds for pharmaceuticals and cosmetics (Jeevanandam et al., 2020). The potential of microalgae in producing value-added lipids, proteins, and other bioactive molecules presents opportunities for developing innovative pharmaceutical and cosmetic formulations (Conde et al., 2021). The bioprospecting of microalgae for biopharmaceutical applications underscores the growing interest in harnessing the therapeutic potentials of microalgae in the pharmaceutical industry (Saeed et al., 2021). In conclusion, microalgae production holds immense promise for the pharmaceutical and cosmetic industries, offering a sustainable and bioactive-rich source for developing a wide range of products. The diverse bioactive compounds synthesized by microalgae present opportunities for formulating novel pharmaceutical drugs, nutraceuticals, and cosmetic products, catering to the evolving needs of these industries.

Biodiesel production

Microalgae production has attracted significant interest as a sustainable and efficient feedstock for biodiesel production, offering a promising solution to the global energy demand

and environmental concerns. The potential applications of microalgae in biodiesel production have been extensively studied, highlighting their viability as a renewable energy source (Ajala et al. 2022). Microalgae possess characteristics that make them ideal for sustainable biodiesel production, including high lipid content and the ability to be genetically optimized for enhanced lipid accumulation (Mohamed et al., 2022).

The utilization of microalgae for biodiesel production involves various advanced technologies and influencing parameters that contribute to maximizing the efficiency of lipid extraction and conversion processes (Cao et al., 2013). The unique properties of microalgae, such as their ability to effectively remove nutrients and heavy metals from wastewater, further enhance their appeal as a biodiesel feedstock (Cobos et al., 2020). Additionally, the direct conversion of lipids within microalgae cells into biodiesel through in situ transesterification has emerged as an effective and simplified approach in biodiesel production (Hidalgo et al., 2015).

The potential of microalgae for biodiesel production extends to their high lipid content, which allows for the generation of saturated and monounsaturated fatty acids ideal for biodiesel synthesis (Mohmmed et al., 2022). The sustainable production of biodiesel from microalgae offers advantages over traditional plant crops, as microalgae can accumulate significant quantities of triglycerides and free fatty acids, making them a valuable resource for biofuel production (Andrade & Andrade, 2018). Furthermore, the joint production of biodiesel and bioethanol from microalgae biomass presents a cost-effective and efficient approach to biofuel production (Sendzikiene & Makareviciene, 2022). The biodiesel potential of microalgae is further underscored by their ability to grow in the presence of sunlight and carbon dioxide, making them excellent sources of biofuels after processing (Ma et al., 2014). The differential fatty acid profiles of microalgae grown with organic materials highlight the versatility of microalgae in biodiesel production and wastewater treatment applications. The integration of oleaginous microalgae cultivation with wastewater treatment is considered a low-cost and sustainable approach for producing algae-based biodiesel.

Biomethane and biogas production

Microalgae production has emerged as a promising avenue for biomethane and biogas production, offering sustainable solutions for renewable energy generation and waste management. The potential applications of microalgae in biomethane and biogas production have been extensively explored, showcasing their versatility as a renewable energy feedstock (Poggio et al. 2023). Microalgae possess unique characteristics that make them ideal for biomethane and biogas production, including their ability to capture CO_2 and convert it into valuable methane gas (Prandini et al., 2016). The integration of microalgae-based wastewater treatment with biogas purification accelerates nutrient removal while producing valuable biomass and biomethane, highlighting the dual benefits of microalgae in waste management and energy production (Ahmad et al., 2014). Furthermore, co-digestion of microalgae with organic waste streams, such as palm oil mill effluent, has been shown to enhance biogas and biomethane production rates, demonstrating the potential of microalgae in optimizing anaerobic digestion processes (Córdova et al., 2018).

The advantages of using microalgae as a substrate for biogas and biomethane production stem from their high productivity, ability to capture CO_2, and convert organic matter into biofuels efficiently (Jensen & Skovsgaard, 2017). The upgrading of raw biogas to biomethane involves removing excess CO2 to produce a higher quality fuel suitable for various applications, such as transportation and heat generation (Machado, 2023). The scalability of anaerobic digestion of microalgae biomass within water resource recovery facilities further emphasizes the potential of microalgae in large-scale biomethane production (Milbrandt et al., 2016). Photosynthetic biogas upgrading has enabled the production of biomethane while concurrently treating digestate in constructed wetlands to obtain biofertilizers, showcasing the integrated approach of utilizing microalgae for energy and environmental benefits (Díez-Montero et al., 2020). The techno-economic analysis of biogas production from microalgae through anaerobic digestion highlights the feasibility and sustainability of biomethane production from this renewable feedstock (Murano et al., 2021). Moreover, modeling studies on hydrogen sulfide removal under biomethane production underscore the importance of optimizing biogas purification processes for efficient biomethane generation (Chernysh et al., 2021).

Bioremediation of air and wastewater

Microalgae have been recognized as a versatile and sustainable solution for bioremediation of air and wastewater, offering a promising approach to environmental cleanup and resource recovery. Extensive research has demonstrated the ability of microalgae to efficiently remove pollutants and contaminants from various environmental matrices (Jalilian et al. 2020; Uguz et al., 2022). Microalgae are well-known for their capacity to thrive in wastewater environments and effectively remediate pollutants, making them valuable assets in the pursuit of cleaner ecosystems and sustainable resource management.

The utilization of microalgae for bioremediation of wastewater presents a win-win paradigm, where the bioremediation capacity of microalgae is harnessed to treat wastewaters, thereby promoting the development of the microalgae industry and offering alternatives to conventional wastewater treatment processes (Delrue et al., 2016). Microalgae-based bioremediation processes have been shown to be cost-effective, energy-efficient, and highly effective in reducing pollutant levels compared to traditional wastewater treatment methods (Singh et al., 2022). The ability of microalgae to assimilate organic nutrients from wastewater for growth while simultaneously reducing pollutant content underscores their dual role in bioremediation and biomass generation (Chew & Show, 2022). Studies have demonstrated that microalgae can effectively remove contaminants from wastewater, leading to a reduction in biological oxygen demand and nutrient levels, thereby contributing to cleaner water sources and sustainable environmental practices (Rawat et al., 2011). The bioremediation potential of microalgae extends to the treatment of various types of wastewaters, including industrial effluents and urban waste, highlighting their versatility in addressing pollution challenges (Mutanda et al., 2020). The integration of microalgae-based bioremediation processes with biofuel production further enhances the sustainability and efficiency of wastewater treatment, offering a holistic approach to environmental stewardship (Renuka et al., 2014). Moreover, the application of microalgae in bioremediation of air and wastewater is not only effective in pollutant reduction but also contributes to biomass production, which can be utilized for various purposes, including biofuel production and nutrient recycling (Miazek et al., 2015). The ability of microalgae to thrive in contaminated environments and remove pollutants, such as heavy metals and organic compounds, underscores their potential as eco-friendly bioremediation agents (Li et al., 2018). The integration of microalgae with other remediation technologies, such as sulfate-reducing bacteria, in continuous treatment systems further enhances the efficiency and effectiveness of bioremediation processes. In conclusion, microalgae offer a sustainable and effective solution for bioremediation of air and wastewater, providing a dual benefit of environmental cleanup and biomass generation. The diverse applications and promising results of microalgae-based bioremediation processes highlight the potential of microalgae in promoting environmental sustainability and resource recovery.

CONCLUSIONS

Microalgae production is at the forefront of a sustainable bio-based economy, with applications in various industries. High lipid content in some microalgae species makes them a promising source for sustainable biofuels, addressing energy demands and environmental concerns. Their bioactive metabolites are increasingly used in pharmaceuticals and cosmetics. Microalgae biomass production provides a cost-effective and eco-friendly source of bioactive compounds for these industries. On the other hand, microalgae offer nutrient-rich alternatives for livestock, poultry, aquaculture feed, and food products, addressing food scarcity and promoting sustainability. Additionally, microalgae serve as feedstock for biomethane and biogas, offering renewable energy solutions and waste management. Integrating microalgae-based wastewater treatment with biogas purification accelerates nutrient removal and biomass production, showcasing dual benefits. Their application in bioremediation of air and wastewater offers sustainable solutions for environmental cleanup, contributing to cleaner ecosystems. In summary, microalgae-based technologies hold significant potential for environmental sustainability, resource recovery, and developing a bio-based economy that addresses energy demand, pollution, and resource management challenges.

REFERENCES

Andrade, C. J. d. and Andrade, L. M. d. (2018). Microalgae for bioremediation of textile wastewater: an overview. MOJ Food Processing &Amp; Technology, 6(5). https://doi.org/10.15406/mojfpt.2018.06.00200

Banerjee, C., Dubey, K. K., & Shukla, P. (2016). Metabolic engineering of microalgal based biofuel production: prospects and challenges. Frontiers in Microbiology, 7. https://doi.org/10.3389/fmicb.2016.00432

Caporgno, M. P. and Mathys, A. (2018). Trends in microalgae incorporation into innovative food products with potential health benefits. Frontiers in Nutrition, 5. https://doi.org/10.3389/fnut.2018.00058

Chaves, A. A. M., Martins, C. d. M. G., Carvalho, D. F. P., Ribeiro, D. M., Lordelo, M., Freire, J. P. B., … & Almeida, A. M. (2021). A viewpoint on the use of microalgae as an alternative feedstuff in the context of pig and poultry feeding—a special emphasis on tropical regions. Tropical Animal Health and Production, 53(3). https://doi.org/10.1007/s11250-021-02800-5

Cobos, M., Gómez, J. C. C., Paredes, J. D., Pérez, S., Maddox, J. D., Estela, S. L., … & Gonzáles, R. I. (2020). Isolation, characterization, and biotechnological potential of native microalgae from the peruvian amazon. Microalgae - From Physiology to Application. https://doi.org/10.5772/intechopen.89515

Hashim, A., Al-Hatim, R.R., Hussein, F. H., Al-Younis, Z.K. (2023). Nutritional value and Functional Properties of Algae Proteins. HNSJ Journal, 4 (3), https://doi.org/10.53796/hnsj4321

Igual, M., Uribe-Wandurraga, Z. N., García-Segovía, P., & Martínez-Monzó, J. (2021). Microalgae-enriched breadsticks: analysis for vitamin c, carotenoids, and chlorophyll a. Food Science and Technology International, 28(1), 26-31. https://doi.org/10.1177/1082013221990252

Luo, X., Su, P., & Zhang, W. (2015). Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications. Marine Drugs, 13(7), 4231-4254. https://doi.org/10.3390/md13074231

Ma, M. and Hu, Q. (2023). Microalgae as feed sources and feed additives for sustainable aquaculture: prospects and challenges. Reviews in Aquaculture, 16(2), 818-835. https://doi.org/10.1111/raq.12869

Moheimani, N. R., McHenry, M. P., & Boer, K. d. (2013). The forefront of low-cost and high-volume open microalgae biofuel production. Applications of Microbial Engineering, 436-459. https://doi.org/10.1201/b15250-19

Mourelle, M. L., Gómez, C. P., & Legido, J. (2017). The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. Cosmetics, 4(4), 46. https://doi.org/10.3390/cosmetics4040046

Saadaoui, I., Rasheed, R., Aguilar, A., Cherif, M., Jabri, H. A., Sayadi, S., … & Manning, S. R. (2021). Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. Journal of Animal Science and Biotechnology, 12(1). https://doi.org/10.1186/s40104-021-00593-z

Sathasivam, R., Radhakrishnan, R., Hashem, A., & Abd_Allah, E. F. (2019). Microalgae metabolites: a rich source for food and medicine. Saudi Journal of Biological Sciences, 26(4), 709-722. https://doi.org/10.1016/j.sjbs.2017.11.003

Shin, S., Lim, J., Koh, H. G., Kim, M. J., Kang, N. H., Jeon, S. G., … & Jeong, B. (2016). Crispr/cas9-induced knockout and knock-in mutations in chlamydomonas reinhardtii. Scientific Reports, 6(1). https://doi.org/10.1038/srep27810

Soto-Sánchez, O., Hidalgo, P., González, A. S., Oliveira, P. E., Hernández, A., & Dantagnan, P. (2023). Microalgae as raw materials for aquafeeds: growth kinetics and improvement strategies of polyunsaturated fatty acids production. Aquaculture Nutrition, 2023, 1-19. https://doi.org/10.1155/2023/5110281

Sui, Y. and Vlaeminck, S. E. (2020). Dunaliella microalgae for nutritional protein: an undervalued asset. Trends in Biotechnology, 38(1), 10-12. https://doi.org/10.1016/j.tibtech.2019.07.011

Udayan, A., Pandey, A. K., Sharma, P., Sreekumar, N., & Kumar, S. (2021). Emerging industrial applications of microalgae: challenges and future perspectives. Systems Microbiology and Biomanufacturing, 1(4), 411-431. https://doi.org/10.1007/s43393-021-00038-8

Ugya, A. Y. and Meguellati, K. (2022). Microalgae biomass modelling and optimization for sustainable biotechnology – a concise review. Journal of Ecological Engineering, 23(9), 309-318. https://doi.org/10.12911/22998993/150627

Vázquez-Villegas, P., Torres-Acosta, M. A., Garcia-Echauri, S. A., Aguilar-Yáñez, J. M., Rito-Palomares, M., & Ruiz-Ruiz, F. (2018). Genetic manipulation of microalgae for the production of bioproducts. Frontiers in Bioscience, 10(1), 254-275. https://doi.org/10.2741/e821

Wu, X., Ruan, R., Du, Z., & Liu, Y. (2012). Current status and prospects of biodiesel production from microalgae. *Energies*, *5*(8), 2667-2682.

Khavari, F., Saidijam, M., Taheri, M., & Nouri, F. (2021). Microalgae: therapeutic potentials and applications. Molecular Biology Reports. https://doi.org/10.1007/s11033-021-06422-w

Kaur, M., Bhatia, S., Gupta, U. S., Decker, E. A., Tak, Y., Bali, M., … & Bala, S. (2023). Microalgal bioactive metabolites as promising implements in nutraceuticals and pharmaceuticals: inspiring therapy for health benefits. Phytochemistry Reviews, 22(4), 903-933. https://doi.org/10.1007/s11101-022-09848-7

Villaró, S., Ciardi, M., Morillas-España, A., Sánchez-Zurano, A., Acién-Fernández, G., & Lafarga, T. (2021). Microalgae derived astaxanthin: research and consumer trends and industrial use as food. Foods, 10(10), 2303. https://doi.org/10.3390/foods10102303

Dussably, J., Mshvildadze, V., Pichette, A., & Ripoll, L. (2022). Microalgae and diatom - potential pharmaceutical and cosmetic resources – review. Journal of Biomedical Research &Amp; Environmental Sciences, 3(9), 1082-1092. https://doi.org/10.37871/jbres1559

Mourelle, M. L., Gómez, C. P., & Legido, J. (2017). The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. Cosmetics, 4(4), 46. https://doi.org/10.3390/cosmetics4040046

Balasubramaniam, V., Gunasegavan, R. D., Mustar, S., Muhammad, H., & Noh, M. F. M. (2021). Isolation of industrial important bioactive compounds from microalgae. Molecules, 26(4), 943. https://doi.org/10.3390/molecules26040943

Morais, M. G. d., Vaz, B. d. S., Morais, E. G. d., & Costa, J. A. V. (2015). Biologically active metabolites synthesized by microalgae. BioMed Research International, 2015, 1-15. https://doi.org/10.1155/2015/835761

Jeevanandam, J., Harun, R., Lau, S. Y., Sewu, D. D., & Danquah, M. K. (2020). Microalgal biomass generation via electroflotation: a cost-effective dewatering technology. Applied Sciences, 10(24), 9053. https://doi.org/10.3390/app10249053

Conde, T., Couto, D., Melo, T., Costa, M., Silva, J., Domingues, M. R. M., … & Domingues, P. (2021). Polar lipidomic profile shows chlorococcum amblystomatis as a promising source of value-added lipids. Scientific Reports, 11(1). https://doi.org/10.1038/s41598-021-83455-y

Saeed, M., Hussain, N., Shahbaz, A., Hameed, T., Iqbal, H. M., & Bilal, M. (2021). Bioprospecting microalgae and cyanobacteria for biopharmaceutical applications. Journal of Basic Microbiology, 62(9), 1110-1124. https://doi.org/10.1002/jobm.202100445

Ajala, E. O., Ajala, M. A., Akinpelu, G. S., & Akubude, V. C. (2022). Cultivation and processing of microalgae for its sustainability as a feedstock for biodiesel production. Nigerian Journal of Technological Development, 18(4), 322-343. https://doi.org/10.4314/njtd.v18i4.8

Mohamed, S., Eladel, H., Battah, M., & Ibrahim, Y. (2022). Screening of the potentiality of four green microalgae to be used as feedstock for biodiesel and nutraceutical production. Egyptian Journal of Phycology, 23(1), 1-33. https://doi.org/10.21608/egyjs.2022.132130.1011

Cao, H., Zhang, Z., Wu, X., & Miao, X. (2013). Direct biodiesel production from wet microalgae biomass ofchlorella pyrenoidosathroughin situtransesterification. BioMed Research International, 2013, 1-6. https://doi.org/10.1155/2013/930686

Cobos, M., Gómez, J. C. C., Paredes, J. D., Pérez, S., Maddox, J. D., Estela, S. L., … & Gonzáles, R. I. (2020). Isolation, characterization, and biotechnological potential of native microalgae from the peruvian amazon. Microalgae - From Physiology to Application. https://doi.org/10.5772/intechopen.89515

Hidalgo, P., Ciudad, G., Schober, S., Mittelbach, M., & Navia, R. (2015). Improving the fame yield of in situ transesterification from microalgal biomass through particle size reduction and cosolvent incorporation. Energy &Amp; Fuels, 29(2), 823-832. https://doi.org/10.1021/ef5023303

Andrade, C. J. d. and Andrade, L. M. d. (2018). Microalgae for bioremediation of textile wastewater: an overview. MOJ Food Processing &Amp; Technology, 6(5). https://doi.org/10.15406/mojfpt.2018.06.00200

Sendžikienė, E. and Makarevičienė, V. (2022). Application of liquid waste from biogas production for microalgae chlorella sp. cultivation. Cells, 11(7), 1206. https://doi.org/10.3390/cells11071206

Ma, Y., Wang, Z., Yu, C., Yin, Y., & Zhou, G. (2014). Evaluation of the potential of 9 nannochloropsis strains for biodiesel production. Bioresource Technology, 167, 503-509. https://doi.org/10.1016/j.biortech.2014.06.047

Poggio, D., Sastraatmaja, A., Walker, M. S., Michailos, S., Nimmo, W., & Pourkashanian, M. (2023). Experimental evaluation of continuous in-situ biomethanation of co2 in anaerobic digesters fed on sewage sludge and food waste and the influence of hydrogen gas–liquid mass transfer. Processes, 11(2), 604. https://doi.org/10.3390/pr11020604

Prandini, J. M., Silva, M. L. B. d., Mezzari, M. P., Pirolli, M., Michelon, W., & Soares, H. M. (2016). Enhancement of nutrient removal from swine wastewater digestate coupled to biogas purification by microalgae scenedesmus spp.. Bioresource Technology, 202, 67-75. https://doi.org/10.1016/j.biortech.2015.11.082

Ahmad, A., Shah, S. S. H., Othman, M. F., & Abdullah, M. (2014). Enhanced palm oil mill effluent treatment and biomethane production by co-digestion of oil palm empty fruit bunches with chlorella sp. The Canadian Journal of Chemical Engineering, 92(9), 1636-1642. https://doi.org/10.1002/cjce.22029

Córdova, O., Chamy, R., Guerrero, L., & Sánchez-Rodríguez, A. (2018). Assessing the effect of pretreatments on the structure and functionality of microbial communities for the bioconversion of microalgae to biogas. Frontiers in Microbiology, 9. https://doi.org/10.3389/fmicb.2018.01388

Jensen, I. G. and Skovsgaard, L. (2017). The impact of co2-costs on biogas usage. Energy, 134, 289-300. https://doi.org/10.1016/j.energy.2017.06.019

Machado, F. L. d. O., Lopes, A. C., Magalhães, G. V. V., Nery de Souza, D., & Stefanutti, R. (2023). Anaerobic co-digestion of microalgae and residual glycerol recovered from biodiesel: evaluation of pretreatment and cod/n ratio. Revista AIDIS De Ingeniería Y Ciencias Ambientales. Investigación, Desarrollo Y Práctica, 614-631. https://doi.org/10.22201/iingen.0718378xe.2023.16.2.81321

Milbrandt, A., Bush, B., & Melaina, M. (2016). Biogas and hydrogen systems market assessment.. https://doi.org/10.2172/1246958

Díez-Montero, R., Vassalle, L., Passos, F., Ortíz, A., García-Galán, M. J., García, J. M. B., … & Ferrer, I. (2020). Scaling-up the anaerobic digestion of pretreated microalgal biomass within a water resource recovery facility. Energies, 13(20), 5484. https://doi.org/10.3390/en13205484

Murano, R., Maisano, N., Selvaggi, R., Pappalardo, G., & Pecorino, B. (2021). Critical issues and opportunities for producing biomethane in italy. Energies, 14(9), 2431. https://doi.org/10.3390/en14092431

Chernysh, Y., Bálintová, M., & Chubur, V. (2021). Modeling of hydrogen sulfide removal under biomethane production in the concept of renewable energy potential growth of ukraine. E3S Web of Conferences, 280, 05001. https://doi.org/10.1051/e3sconf/202128005001

Jalilian, N., Najafpour, G., & Khajouei, M. (2020). Macro and micro algae in pollution control and biofuel production – a review. ChemBioEng Reviews, 7(1), 18-33. https://doi.org/10.1002/cben.201900014

Delrue, F., Álvarez-Díaz, P., Fon-Sing, S., Fleury, G., & Sassi, J. (2016). The environmental biorefinery: using microalgae to remediate wastewater, a win-win paradigm. Energies, 9(3), 132. https://doi.org/10.3390/en9030132

Singh, A., Shourie, A., & Mazahar, S. (2022). Integration of microalgae-based wastewater bioremediation–biorefinery process to promote circular bioeconomy and sustainability: a review. CLEAN – Soil, Air, Water, 51(3). https://doi.org/10.1002/clen.202100407

Chew, K. W. and Show, P. L. (2022). Adapting microalgae-based strategies for sustainable green cities. Biotechnology Journal, 17(10). https://doi.org/10.1002/biot.202100586

Rawat, I., Kumar, R. R., Mutanda, T., & Bux, F. (2011). Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Applied Energy, 88(10), 3411-3424. https://doi.org/10.1016/j.apenergy.2010.11.025

Mutanda, T., Naidoo, D., Bwapwa, J. K., & Anandraj, A. (2020). Biotechnological applications of microalgal oleaginous compounds: current trends on microalgal bioprocessing of products. Frontiers in Energy Research, 8. https://doi.org/10.3389/fenrg.2020.598803

Renuka, N., Sood, A., Prasanna, R., & Ahluwalia, A. S. (2014). Phycoremediation of wastewaters: a synergistic approach using microalgae for bioremediation and biomass generation. International Journal of Environmental Science and Technology, 12(4), 1443-1460. https://doi.org/10.1007/s13762-014-0700-2

Miazek, K., Iwanek, W., Remacle, C., Richel, A., & Goffin, D. (2015). Effect of metals, metalloids and metallic nanoparticles on microalgae growth and industrial product biosynthesis: a review. International Journal of Molecular Sciences, 16(10), 23929-23969. https://doi.org/10.3390/ijms161023929

Li, Y., Xiao-hu, Y., Geng, B., & Xue, L. (2018). Effective bioremediation of cu(ii) contaminated waters with immobilized sulfate-reducing bacteria-microalgae beads in a continuous treatment system and mechanism analysis. Journal of Chemical Technology &Amp; Biotechnology, 93(5), 1453-1461. https://doi.org/10.1002/jctb.5513

IDENTIFICATION AND MAPPING OF NON-AGRICULTURAL AREAS USING REMOTE SENSING METHODS: THE HARRAN PLAIN IN TURKEY

Mahmut DİNGİL[1]

Çukurova University, AOSB Vocational School of Technical Sciences, Department of Architecture and Urban Planning, GIS Program, Adana, Turkey

Corresponding author e-mail: mdingil@gmail.com

ABSTRACT

The study was conducted in the Harran Plain (HP), which has high agricultural potential. No severe natural factors limit agricultural activities in the study area. This study aims to determine the uses that cause the loss of agricultural lands in the Harran Plain and to determine the distribution and percentage ratios of these areas within the plain. Among these uses, settlements (CS; Current Settlements and HHS; Historical Harran Structures) and the region used as a Military Border Zone (MBZ) were considered. It has been determined that the non-agricultural area of the study area, which has a surface area of 157,252 ha, is 6474.04 ha due to CS, HHS, and MBZ. This amount means that approximately 4.11% of the Harran Plain is taken out of agriculture by the people. These lands, which were taken out of agriculture, were formed not because of misuse but because of basic needs. Moreover, lands outside agriculture caused by road networks formed over time in the study area are not included in this value. However, another data revealed in the interpretation of the satellite image is that the excessive and unplanned road network also reduces the usage areas of agricultural lands in the plain. The results show that remote sensing methods using up-to-date satellite imagery play an important role in revealing the distribution, amount, and causes of lands taken out of agricultural use in areas where intensive agricultural activities are applied.

Keywords: Remote sensing, non-agricultural mapping, land use, Harran Plain.

INTRODUCTION

38% of Turkey's surface area consists of lands with a slope of 0-12% and 62% consists of lands with a slope of 12-30%+. Only 13% of the 38% consists of flat/near-flat lands (0-2%) (Topçu, 2012). In places where the topography has such a distribution, the importance of arable and flat areas increases. The study area is a very fertile plan and is in this 13% valuable flat/near-flat area. Agricultural lands in the study area are no longer used for agricultural purposes because of human-induced misuse and unplanned use. Therefore, there is a loss of agricultural areas in the Harran Plain. It is possible to encounter the same problem in different geographies.

The researchers found that change detection analysis in Mansoura and Talkha during 25 years shows that the built-up area has increased from 28 to 255 km2 by more than 30% and agricultural land has reduced by 33% (Hegazy and Kaloop, 2015). The image characteristics on the satellite data of the Harran Plain and Mansoura and Talkha are very similar in terms of land use, shape, and area size. In a study conducted in Sivas province, they found that the city developed in the northeast, south, and southwest directions (Karakuş et al., 2015). Many lands are facing different problems like soil erosion, water logging, groundwater depletion, heavy runoff, and productivity losses. (Barah, 2010; Zolekar and Bhagat, 2014). Urban sprawl is one of the main problems that threaten the limited highly fertile land in the Nile Delta of Egypt

(Shalaby, 2012; Shalaby et al., 2012). In a study conducted between 1992 and 2009, it was determined that the total available agricultural areas decreased by 43.5% compared with the total area, whereas urban use increased to 202.5% within the same period and area (Afifi et al., 2013). In a study conducted in the Nile Delta, while the rate of loss of agricultural lands to urban use was high between 1984 and 1992, urban development was directed to lands that were not suitable for agriculture between 1992 and 2006 (Shalaby, 2012). In a study in Nigeria, the researchers found that the percentage changes in arable lands, bare surface lands, settlements, forests, and water bodies were 11.24%, -5.86%, 28.36%, -33.87%, and 0.13%, respectively (Balogun et al., 2011). Several studies have shown that unplanned changes in land use due to urbanization have become a major problem (Zhao et al., 2004; Nanda, 2005). In a study conducted between 1987 and 2001 on the northwestern coast of Egypt, a serious land cover change occurred because of agricultural and tourism development projects, vegetation deteriorated in a part of the study area, and water accumulation areas emerged (Shalaby and Tateishi, 2007).

Remote sensing and GIS approaches were used to identify land use and land cover change between 2002 and 2022 in the Mymensingh region of Bangladesh, which is a rapidly developing and growing region. Researchers found that over a 20-year period, the biggest change was in construction land use, resulting from the expansion of the Bhaluka town area by 217.1% (6.56 km2) (Seyam et al., 2023). Similarly, another important finding found in another studies are that the growth rate of urbanization and population growth are among the main factors that cause cultivated areas to become non-agricultural (Ding et al., 2022; Abebe et al., 2023).

In this study, we aimed to determine the current land uses that caused the loss of agricultural lands in the Harran Plain using remote sensing and to determine the percentages by mapping the distribution of these areas within the plain.

MATERIAL AND METHOD

The study area is a very fertile plain in the southeastern Anatolia Region of Turkey (Figure 1). It is located between the coordinates $37^{o}11^{'}59^{''}$ N, $38^{o}47^{'}58^{''}$ E and $36^{o}39^{'}54^{''}$ N, $36^{o}11^{'}59^{''}$ E and is adjacent to Syria. The geological formation of the study area is a graben plain. HP has plenty of settlements (mostly small) in 157.252 ha. Apart from these residential areas, there is a historical site in the middle of the plain where the old Harran houses are located. The study area has a bowl-shaped slope in the west-east direction and a slight slope toward the Syrian border in the North-South direction (Figure 2). Therefore, the Harran Plain has very fertile soils.

Google Earth satellite imagery was used to identify land uses in the study area. The resolution of the image is highly suitable for this interpretation. QGIS 3.16.2 was used for digitizing and GIS processes (geo-referencing, labeling, mapping, etc). Necessary proportional calculations were performed using Microsoft Excel.

Figure 1. Location map of the study area (Harran Plain).

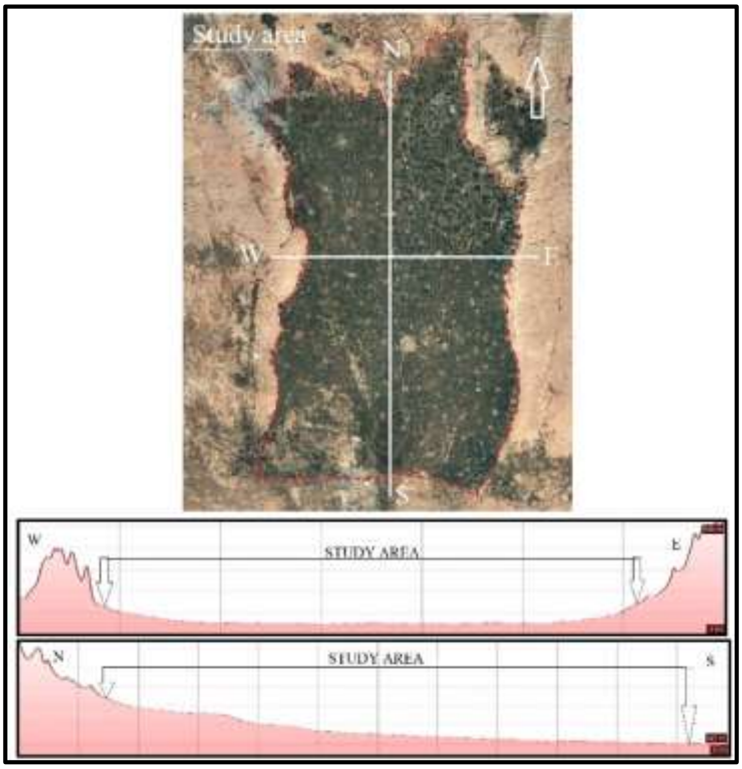


Figure 2. On top; directions of cross sections (North-South, and East-West) on the satellite image (Google Earth) and below; its cross-section profiles of the study area.

Methodology; five basic stages were applied in this study. The study area was identified in the first stage. The Harran Plain (HP) was selected as the study area. The second stage includes downloading satellite imagery of the study area and its geo-referencing process. The coordinates required for the control points to be used in the geographic correction of the satellite image were obtained from Google Earth satellite data. The third stage is the interpretation of the satellite imagery. Roads, irrigation cannels, HHS, CS, and MBZ were defined as a result of image interpretation. Digitization of identified land use types is the fourth stage of this study.

The borders of the study area, historical settlements, residential areas, and mined fields were digitized. We focused on these uses in the agricultural fields of HP. The data obtained according to the results of the research findings were also evaluated in the fifth stage, which constitutes the last stage of the study. The workflow of the applied methodology is shown in Figure 3.

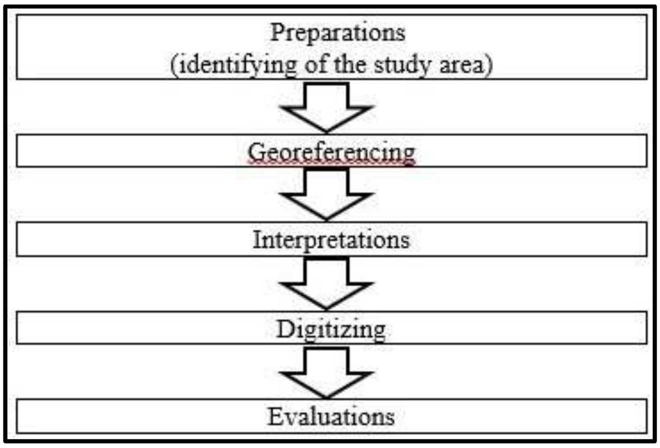


Figure 3. Workflow of the applied methodology in the study.

RESULTS AND DISCUSSION

The study area is approximately 155,252 ha. It is mostly used for agricultural activities. However, some agricultural areas have irregular shapes and some small sizes because of traditional reasons. There are too many roads between agricultural fields and settlements. While it is desired that the agricultural lands be adjacent to the road at least at one border, this situation is also seen in the second and even third borders in the study area. This is a factor that causes the loss of agricultural lands in the HP. These positions of the roads were not evaluated in this study.

In the image interpretation, it was determined that the main land use types in the study area were CS (5518.17 ha), HHS (69 ha), and MBZ (886.87 ha), with the exception of roads. These land use types are shown in Figure 4. The spread of CS, HHS, and MBZ in the study area was 3.51, 0.04, and 0.56%, respectively (Figure 5). In other words, 6,474.04 ha of the study area (4.11 %) has been excluded from agricultural use because of the defined uses.

A closer look at the area where the HHS is located reveals that this historic settlement is surrounded by CS (Figure 6). This close location also increases the risk of damage to the HHS tissue.

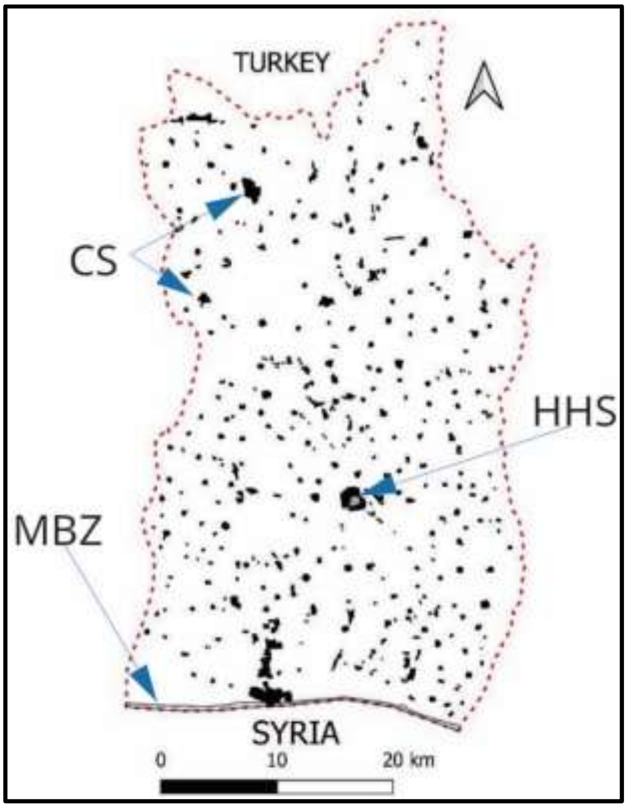


Figure 4. Distributions of CS (all of the black pieces), HHS, and MBZ in the study area.

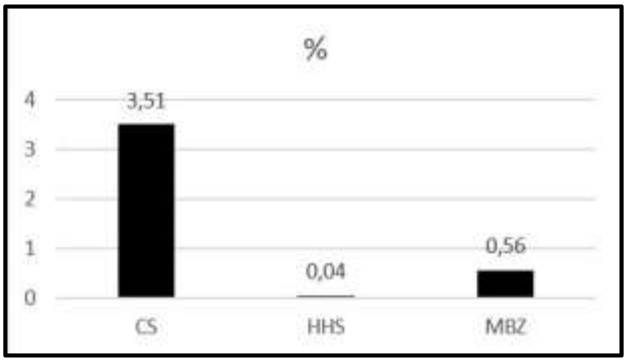


Figure 5. Distribution rates of land use (CS; Current Settlement, HHS; Historical Harran Settlement, MBZ; Military Border Zone) causing loss of agricultural area within the study area.

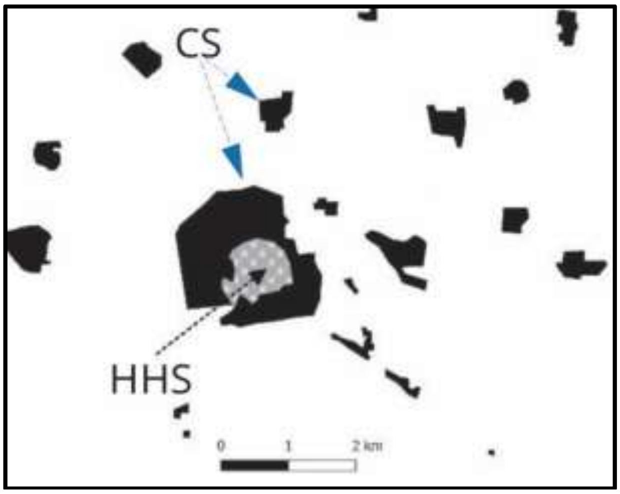


Figure 6. Black areas show CS, gray area shows HHS in the study area (a more detailed view).

It has been determined that a significant amount of agricultural land has been lost in a way that cannot be recycled because of unplanned and incorrect land use in the study area. Considering the road network used for transportation in the Harran Plain, the loss of agricultural lands has increased.

CONCLUSIONS

In a previous study, land uses were successfully determined, and the findings were mapped using remote sensing methods. In the other conducted research, it was observed that agricultural lands decreased over a 25-year period (decreased by 33%), and this reduction was attributed to the expansion of residential areas (increased by 30%). In a study conducted between 1992 and 2009 (19-years period), it was determined that the total available agricultural areas decreased by 43.5% compared with the total area, while urban use increased to 202.5% within the same period and area. In previous studies, positive/negative changes in agricultural and residential areas over the years have been assessed. However, in this study, the current distributions of land use types causing the loss of agricultural areas have been evaluated. In both cases, the factors leading to the loss of agricultural areas exhibited similarities.

In conclusion, the results of this research demonstrated that the study area has three main reasons for the loss of agricultural area. These are settlements (CS and HHS), military zones, and road networks in the Harran Plain. It is predicted that the irregularity of the settlements is caused by culture, and the military zone is due to the geopolitical position. The road network also exhibits a distribution that is dependent on the spread of settlements within the study area. In other words, the arrangement of settlements and the pattern of the road network are directly proportional to each other. If we make a comparison; considering that a soccer field is approximately 0.71 ha, there is a loss of land suitable for agricultural use in the size of approximately 9021 soccer fields in the study area.

The factors that cause loss of agricultural land are not the same in different environments and cultures; these factors may vary. MBZ, which is an important factor in our study, may not be important in another agricultural area. The effect of road networks on the loss of agricultural land increases in direct proportion to the irregularity and density of settlements. Complex road network characteristics are also the result of irregular settlements. The next step in the future should be the assessment of the unplanned road network and the changes in the loss of agricultural areas in Harran Plain from the past to the present.

Furthermore, this study has the potential for further investigations, particularly in assessing the impact of unplanned road networks on agricultural land loss. As technology continues to advance, these methods offer a pathway for creating informed land management strategies that balance the demands of urbanization with the preservation of vital agricultural areas. Overall, this study contributes to a broader understanding of landscape changes, offering insights that can inform sustainable land use planning and resource management practices.

REFERENCES

Abebe B G, Gemeda B S, Sisay G, 2023. Analysing land use land cover (LULC) dynamics by using remote sensing and GIS techniques: the case of Dukem town, Oromia special zone. Geocarto International, 38:1. https://doi.org/10.1080/10106049.2023.2210523

Afifi A, Elsemary M A, Wahab M A, 2013. Urban sprawl of greater Cairo and its impact on the agricultural land using remote sensing and digital soil map. Journal of Applied Sciences Research, 9(8): 5159-5167.

Balogun I A, Adeyewa D Z, Balogun A A, Morakinyo T E, 2011. Analysis of urban expansion and land use changes in Akure, Nigeria, using remote sensing and geographic information system (GIS) techniques. Journal of Geography and Regional Planning Vol. 4 (9), pp. 533-541.

Barah B C, 2010. Hill agriculture: problems and prospects for mountain agriculture. Ind. J. Agric. Econo. 65 (3), 584–601

Ding S, Li M, Wang X, Li L, Wu R, Heng H, 2022. The Use of Time Series Remote Sensing Data to Analyze the Characteristics of Non-agriculture Farmland and Their Driving Factors in Fuzhou. Remote Sensing Technology and Application, 37(3): 550-563 https://doi.org/10.11873/j.issn.1004-0323.2022.3.0550 (in Chinese)

Hegazy İ R, Kaloop M R, 2015. Monitoring urban growth and land use change detection with GIS and remote sensing techniques in Daqahlia governorate Egypt. International Journal of Sustainable Built Environment, 4, 117-124. https://doi.org/10.1016/j.ijsbe.2015.02.005

Karakuş C B, Cerit O, Kavak K S, 2015. Determination of land use/cover changes and land use potentials of Sivas city and its surroundings using GIS and RS. Procedia Earth and Planetary Science, 15, 454-461. https://doi.org/10.1016/j.proeps.2015.08.040

Nanda T, 2005. Urban Sprawl and Occupational Change in Raipur City. Unpublished MSc Thesis, Department of Geography, University of New Delhi, Delhi.

Seyam M M H, Haque M R, Rahman M M, 2023. Identifying the land use land cover (LULC) changes using remote sensing and GIS approach: A case study at Bhaluka in Mymensingh, Bangladesh. Case Studies in Chemical and Environmental Engineering, 7, 100293. https://doi.org/10.1016/j.cscee.2022.100293

Shalaby A, Tateishi R, 2007. Remote sensing and GIS for mapping and monitoring land cover and land-use changes in the Northwestern coastal zone of Egypt. Applied Geography, 28-41. https://doi.org/10.1016/j.apgeog.2006.09.004

Shalaby A, 2012. Assessment of Urban Sprawl Impact on the Agricultural Land in the Nile Delta of Egypt Using Remote Sensing and Digital Soil Map. International Journal of Environmental Sciences, Vol. 1 No. 4. 2012. Pp. 253-262.

Shalaby A, Ali R R, Gad A, 2012. Urban sprawl impact assessment on the agricultural land in Egypt using remote sensing and GIS: a case study, Qalubiya Governorate. Journal of Land Use Science, Vol. 7, No. 3, 261–273. https://doi.org/10.1080/1747423X.2011.562928

Topçu P, 2012. Policies for the effective use and protection of agricultural lands (Specialization Thesis). General Directorate of Economic Sectors and Coordination. Republic of Turkey Ministry of Development Publication, No: 2836. ISBN: 978-975-19-5771-4 (in Turkish).

Zhao L M, Dickson R E, Tian Y H, 2004. Evidence for a significant urbanization effect on urban climate in China. P Natl. Acad. Sci. USA, 101: 9540- 9544.

Zolekar R B, Bhagat V S, 2014. Use of IRS P6 LISS-IV data for land suitability analysis for cashew plantation in hilly zone. Asian J. Geoinform, 14 (3), 23–35.

DETERMINATION OF TOTAL PHENOLIC AND TOTAL FLAVANOID CONTENT of *Verbascum myrianthum* and *Astragalus tokatensis* PLANTS

Sibel Bayıl[1], Elif Sine Düvenci[2], Bedrettin Selvi[3]

[1] *Gaziantep University, Science Faculty, Department of Biology, Gaziantep, Türkiye*
[2]*Düzce University, Scientific and Technological Research Application and Research Center, Düzce, Türkiye*
[3] *Gazi Osman Paşa University, Faculty of Arts and Sciences, Department of Botany, Tokat, Türkiye*
Corresponding author e-mail: bayil@gantep.com.tr

ABSTRACT

The present study aimed to investigate the total phenolic and total flavonoid amounts of two distinct endemic species, namely Astragalus tokatensis Fischer and Verbascum myrianthum Boiss. A review of the literature revealed no studies on the bioactive compounds of these two plants. Consequently, methanol and hexane extracts of the flowers and leaves of the two plants were obtained. The flavonoid and phenolic amounts of the extracts obtained in different solvents were determined and compared with one another. The findings demonstrated that both Astragalus tokatensis Fischer. and Verbascum myrianthum Boiss. possess flavonoid and phenolic active compounds. The findings indicate that the total phenolic substance measurements were higher in the methanolic extracts of Verbascum myrianthum and Astragalus tokatensis plants than in the ethanolic extracts. In the case of the Astragalus tokatensis plant, the total flavonoid content was found to be similar in both the methanolic and ethanolic extracts.

Keywords: *Astragalus tokatensis Fischer*, *Verbascum myrianthum* Boiss., flavonoids, and phenolics.

INTRODUCTION

Phenolic compounds are defined as compounds containing one or more hydroxyl groups directly attached to the aromatic ring (Vermerris et. Al 2006). Phenolic substances are of great importance for both the plants themselves and for humans when plants are consumed. Phenolic substances are known to possess a multitude of properties, including antioxidant properties. Antioxidant substances are capable of suppressing the formation of free radicals. Consequently, a significant number of studies have concentrated on the antioxidant properties of phenolic substances. Research has demonstrated that antioxidant substances have a beneficial impact on degenerative diseases, cardiovascular diseases and, in particular, cancer (Murkovic et al). Consequently, the determination of the total amount of phenolic substances in plants has become a crucial area of interest.

Flavonoids are currently employed in a multitude of fields, particularly in the pharmaceutical, medical, and cosmetic sectors. Flavonoids, which are found in diverse plant parts, are regarded as significant natural products due to their polyphenolic structures (Panche et. Al 2016).

The determination of phenolic matter was modified and developed by Folin and Ciocalteu in 1927 and has since become the most widely used method for the determination of total phenolic matter. Despite this, there are still hundreds of plants whose total phenolic matter content has not been determined, despite the fact that the determination of phenolic matter can be performed for approximately a century.

Flavonoids are a subclass of phenolic substances. They are found in a variety of vegetables and fruits. The most well-known flavonoids are quercetin and rutin. Studies have demonstrated that flavonoids possess antioxidant properties, whereby they neutralise free radicals. Subsequent studies have revealed that flavonoids have beneficial effects in the treatment of certain diseases, including inflammation, viral infections and cancer (Duangjai et al 2018).

Currently, there are numerous products on the market that claim to be antioxidant-effective. Recently, there has been a notable surge in demand for such antioxidant-containing products. However, due to concerns about the synthetic nature of these products, there has been a shift towards natural alternatives.

The aim of our study was to determine the total phenolic and total flavonoid content in the leaves, buds and flowers of two plant species: Astragalus tokatensis Fischer and Verbascum myrianthum Boiss.

The genus Verbascum is a member of the family Scrophulariaceae, which comprises 360 species distributed worldwide. The species in this genus are typically found in the temperate climates of the northern hemisphere, occupying dry, rocky, and open habitats (Heywood, 1979; Juan et al., 1997). In Turkey, Verbascum is known by the vernacular names ‘mullein’ and ‘king's candlestick’, and 185 of the 233 species are endemic (Davis, 1978; Davis et al., 1988; Güner et al., 2000). Verbascum myrianthum Boiss. is a biennial or perennial species, reaching a height of 60-100 cm. It is characterised by a dense greyish pubescence, which transitions to white pubescence, and an early deciduous habit. The plant becomes glabrous at maturity above. The stem is broadly angular and typically branched from the base. The basal leaves are oblong-angular-lanceolate, measuring 15-20 x 4-6 cm. They are coarsely and bluntly notched, or more often with mid-carved lobes. The margin has carved lobes that are notched or with shallowly lobed segments below. The petiole is 3-5 cm long and the upper petiole is short and subentire. The inflorescence is markedly branched and broadly paniculate, comprising one to four loose flower clusters. The bract (or flower petal) is acutely to ribbony in shape. The peduncle measures 7 mm, and the bracts are narrow and small. The calyx measures 3-6 mm and comprises lobes that are ribbed or ribbed oblong, with blunt tips. The corolla is yellow in colour, with a diameter of 12-16 mm, and is characterised by the presence of transparent glands and external hairs. The plant bears five stamens and spikelets that are reniform in shape. The phyllaries are coated in whitish-yellow hairs, and there are two spikelets that are hairless above. The capsule is elliptic ovate, measuring 4-5 x 3-4 mm, and is characterised by a hairy texture (Davis, 1978; Seçmen et al., 2018). Verbascum species have a long history of use in traditional medicine in their regions of origin and remain in use today. Verbascum species belonging to the Scrophulariaceae family have been employed for centuries in the treatment of various internal and external infections. The majority of communities in Europe, Asia, Africa and North America have also documented the utilisation of the leaves and flowers of Verbascum species, which contain a range of active constituents, for a variety of purposes (Meurer-Grimes et al., 1999).

The Fabaceae is one of the largest families of flowering plants, representing the third largest angiospermae family in the world in terms of number of species. It is surpassed in this regard only by the Asteraceae (Compositae) and Orchidaceae, with nearly 770 genera and more than 19,500 species. In economic terms, it is the second largest family after Poaceae (LPWG, 2017). A total of 69 genera and 958 species have been identified within our country (Seçmen et al., 2018). Astragalus tokatensis Fischer is an endemic species of dwarf cushion-forming shrub, measuring approximately 10 cm in height. The leaves are characterised by the presence of hairs and spines on the petioles. The leaves are 4–6 cm long, oblique, and flattened towards the base. The leaflets are 10-14 mm in length, ovate in shape, mucronate, and simple white tomentellous, with five to six parts. The leaflets, or stipules, are located at the base of the petiole and measure approximately 8 mm. They are ovate to obtuse in shape and sparsely hairy when young, but become glabrous with age. The flowers are sessile, occurring in numbers of three to five in the axils of the leaves. The inflorescence is ovate in shape and approximately 3.5 cm in diameter, comprising 50 or more flowers. The bracts, which are scalelike leaves, are approximately 9 mm long, lanceolate, navicular, and pubescent towards the apex. The bracts measure 6-7 mm and exhibit hairiness towards the apex, while the lower surface is glabrous. The calyx is 10-13 mm in length and is densely hairy, with lobes that are more or less divided at the base. The colour of the corolla is unknown; the standard is 18-20 mm. The flower has a diameter of 7 mm. It is found in steppe habitats at an altitude of approximately 1300 m. These findings were reported by Davis (1978), Davis et al. (1988), and Güner et al. (2000).

MATERIAL AND METHOD

The total phenolic and total flavonoid contents were determined in plants of the species *Verbascum myrianthum* (1) and *Astragalus tokatensis* (2). The plants were subjected to a 15-day drying process in a shaded environment, after which they were separated and subjected to a second drying process in an oven maintained at 40°C. The moisture content of the plants was then determined.

Sample preparation: In total phenolic and flavonoid process, the plants were ground after drying and subjected to both ethanolic and methanolic extraction experiments. In these experiments, the solvent was applied at a ratio of 1:15, and the extraction process was conducted in an ultrasonic bath for six hours. The separated plants were assigned distinct codes.

 **a**	 **b**
 **c**	 **d**

Figure 1. a) Leaves of the plant *Verbascum myrianthum* b) Seeds of the plant *Verbascum myrianthum* c) Leaves of *Astragalus tokatensis* d) Flowers of the plant *Verbascum myrianthum*

Table 1. Coding of analysed samples

Plants	**Leaf**	**Seed**	**Flower**
***Verbascum myrianthum* (methanolic extract)**	1MY	1MT	1MC
***Verbascum myrianthum* (ethanolic extract)**	1EY	1ET	
***Astragalus tokatensis* (methanolic extract)**	2M		
***Astragalus tokatensis* (ethanolic extract)**	2E		

Total Phenolic Substance Determination Method

The total phenolic content was determined using the Folin-Ciocalteu reagent (FCR) method. A Shimadzu UV 1800 Spectrophotometer was utilized for the analysis, with gallic acid (GA) employed as the reference standard. The results were expressed as gallic acid equivalents (GAE). The Folin reagent was prepared at a concentration of 0.5 N. To quantify the phenolic content in terms of mg GA per gram of sample, a stock solution of gallic acid was prepared and subsequently diluted to produce a series of standard solutions. These standards were derived from a 1000 ppm GA stock solution and included concentrations of 500, 250, 125, 62.5, 31.25, and 15.625 ppm. A 10% sodium carbonate (Na_2CO_3) solution was used as the colorimetric indicator. Upon preparation of these solutions, the pipetting process commenced. Total phenolic matter concentration was found by reading the results at 760 nm wavelength.

Determination of Total Flavonoid Substance

Total flavonoid content is determined by $AlCl_3$ colourimetric method. Based on quercetin (QE) standard, it is analysed according to quercetin equivalence. Total flavanoid content was determined by using Shimadzu UV 1800 Spectrophotometer. The absorbance was made by photometric method at 415 nm. For the standard, 1000 ppm stock quercetin (QE) standard was prepared, and 6 different standards were obtained by serial dilution as 250; 125; 62.5; 31.25; 15.625 ppm. 2% $AlCl_3$ was prepared as reagent. Pipetting was performed by adding 2% $AlCl_3$, water, sample in certain ratios to the balconies prepared for pure water, standard and sample. It was kept in the dark for 60 minutes. Total flavonoid matter concentration was found by reading the results at 415 nm wavelength.

RESULTS AND DISCUSSION

In our study, the plants were subjected to a 15-day period of drying in a shaded environment, followed by a second drying process in a 40°C oven. Subsequent to the process of dehydration, measurements of moisture content were conducted. The seeds of Verbascum myrianthum exhibited a moisture content of 8.45%, the flowers displayed 8.72%, and the leaves demonstrated 9.25% moisture content. In contrast, the leaves of Astragalus tokatensis exhibited a moisture content of 7.41%. Subsequently, the harvested plants were diluted in a 1:15 ratio with a solvent. The solvent employed was a methanol-ethanol mixture, and the sample was subjected to an ultrasonic-assisted extraction process for a period of six hours. The total phenolic and flavonoid content assays were conducted in sequential steps, following the prescribed methodology. The Shimadzu UV 1800 spectrophotometer was employed to obtain the appropriate absorbance values.

The results of the determination of total phenolic matter in the leaf parts of the Verbascum myrianthum plant yielded the following values: 255,623 mg GA/L in the methanolic extract (1MY) and 143,582 mg GA/L in the ethanolic extract (1EY). In the determination of total flavonoid content, the methanolic extract exhibited a value of 198,666 mg QE/L, while the ethanolic extract demonstrated a value of 138,582 mg QE/L.
The total phenolic content in the bud parts of the Verbascum myrianthum plant was recorded as 820,547 mg GA/L in the methanolic extract (1MT) and 546,773 mg GA/L in the ethanolic extract (1ET). Upon subjecting the same methanolic and ethanolic extracts to total flavonoid determination, the methanolic extract yielded 197,271 mg QE/L, while the ethanolic extract yielded 179,416 mg QE/L.

In the ethanolic extracts of the yellow flowered (1MC) parts of the same plant, the measurement of total phenolic matter was read as 338.963 mg GA/L, while the total flavanoid amount was read as 144.03 mg QE/L.

The application of the total phenolic matter determination process to the methanolic extract of Astragalus tokatensis yielded a concentration of 415,415 mg GA/L, whereas the ethanolic extract resulted in a concentration of 247,404 mg GA/L. With regard to total flavonoid matter, the methanolic extract exhibited a concentration of 246,543 mg QE/L, while the ethanolic extract demonstrated a concentration of 255,567 mg QE/L.

The findings revealed that the total phenolic substance measurements were higher in the methanolic extracts of *Verbascum myrianthum* and *Astragalus tokatensis* plants than in the ethanolic extracts. In *Astragalus tokatensis*, the total flavonoid content was found to be similar in both methanolic and ethanolic extracts.

Table 2. Total phenolic matter and total flavonoid matter amounts

Numune	**Concentration mg GA/L Total Phenolic Substance**	**Concentration mg QE/L Total Flavanoid Substance**
1MY	255,623	198,666
1MT	820,547	197,271
1MC	338,963	144,03
1EY	143,582	138,582
1ET	546,773	178,416
2M	415,415	246,543
2E	247,404	255,567

CONCLUSIONS

Endemic plants are defined as plant species that are unique to a particular geographical region and do not occur naturally outside that region. These species are typically plants that have adapted to specific ecological conditions and are unable to survive in other regions. An understanding of the ways in which endemic species adapt to specific geographical and climatic conditions is crucial for the study of adaptation mechanisms. These studies yield valuable insights into the genetic diversity of species and the evolutionary processes that shape them. A significant number of endemic plants are utilized in traditional medicinal practices and are also of value for the development of new pharmaceutical agents in modern medicine. The conduct of experimental studies enables the investigation of the pharmacological effects and the medicinal potential of these plants. Phenolic substances and flavonoids have been demonstrated to possess robust antioxidant properties. These compounds serve to safeguard cells against oxidative stress by neutralizing free radicals. The quantification of these compounds in endemic plants is a crucial aspect in the evaluation of the antioxidant capacity and potential health benefits associated with the plant.

REFERENCES

AN. Panche, A. D. Diwan,ve S. R. Chandra1, Flavonoids: an overview, Journal of NutritionalScience (2016), vol. 5, e47, page 1 of 15

Davis, P.H., (1978). Scrophulariaceae. Flora of Turkey and The East Aegean Island. Edinburg: Edinburg University Press, Vol 6: 458-603.

Davis, P.H., Mill, R.R., Tan, K., (1988). Flora of Turkey and The East Aegean Island, Edinburg: Edinburg University Press, Vol10:191-193.

Davis, P. H. (ed.) (1970). Flora of Turkey and the East Aegean Islands, Vol. 3, Edinburgh: Edinburgh University Press.

Duangjai Tungmunnithum, Areeya Thongboonyou, Apinan Pholboon ve Aujana Yangsabai,'FlavonoidsandOtherPhenolicCompoundsfromMedicinalPlantsforPharmaceuticalandMedicalAspects: An Overview' 25 August 2018.

Güner, A., Özhatay, N., Tuna, E., Başar, K.H.C., 2000. Scrophulariaceae. Flora of Turkey and Aegean Islands. (Suplement 2) Edinburg: Edinburg University Press, Vol 11: 193.

Heywood, V.H., 1979. Flowering Plants of the word. Oxford: Oxford University.

Juan, R., Fernandez, I., Pastor, J., 1997. Systematic cansideration of microcharacters of fruit and seeds in the genus Verbascum (Scrophulariaceae). Annals of Botany, 80:591-598.

LPWG - The Legume Phylogeny Working Group. (2017). A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. Taxon, 66(1), 44-77.

M Murkovic,Phenolic Compounds, Graz University of Technology, Graz,Austria sf. 4507

Seçmen, Ö., Gemici, Y., Görk, G., Bekât, L. ve Leblebici, E. (2018). Tohumlu Bitkiler Sistematiği (10. Baskı). Ege Üniversitesi Fen Fakültesi Kitaplar Serisi No: 116, İzmir.

Vermerris W.,Nicholsan R., PhenolicCompoundBiochemistry, Springer, 2006, sf. 2-7

EFFECTS OF BIOSOL BIOFERTILIZER ON THE PRODUCTIVITY AND CHANGE OF HERBAGE IN NATURAL PASTURES IN STRANDZHA MOUNTAIN.

Dimitrinka Krusheva

Experimental Station of Agriculture, 8300, Sredets, Bulgaria
Corresponding author e-mail: kasidi4@abv.bg

ANNOTATION

This article presents the results of a study on the impact of BIOSOL biofertilizer on the productivity and change of the herbage in natural pastures in Strandzha Mountain. Fertilization is the key factor for boosting the productivity and quality of biomass. For sustainable productivity and quality of biomass from grass associations, the most sensible approach is the application of appropriate biological fertilizers. Observations were made on 4 natural grasslands, fertilized with BIOSOL in the area near the state border in South-Eastern Bulgaria (Strandzha Mountain), during the period 2022-2024. The study results provide evidence that fertilization with BIOSOL biofertilizer on natural pastures in the region of Strandzha leads to increase in the yield of green mass by 7-8%. After fertilization, there are changes in the height of the plants in the pasture; the content of crude protein in the dry mass also increased. Botanical composition and herbage density were not affected by fertilization with BIOSOL.

Key words: Strandzha, natural pastures, productivity, biofertilizer

INTRODUCTION

Natural pastures are of quite significant economic and ecological importance, they are the main source of grass protein-rich fodder. The use of natural pastures is the traditional way of feeding animals in the Strandzha Mountain area. One of the main priorities today is the full utilization of the available natural resources, while at the same time emphasizing their preservation. In order to thrive in the future, natural ecosystems require conservation, restoration and proper management *(Godwin et al., 1998; Sutherland et all., 1995).* Integral part of these ecosystems are natural meadows and pastures which are the main source of ecologically clean fodder *(Stoeva et al., 2005).*

Natural pastures in Bulgaria produce 700-1200 kg/ha of hay *(Pavlov, 2007).* Fertilization is the key factor for boosting the productivity and quality of biomass.Studies in Bulgaria show that fertilizing with mineral fertilizers has a strong impact by increasing yields of green fodder and improving the quality of composition of existing natural pastures. (*Boikov. S., 1986; Owen, O., 1981; Totev and Valkov, 1988; Totev, T., 1984; Yakimova, Ya., et al., 1977).*

Conducted studies prove that fertilizing with mineral (nitrogen, phosphorus and potassium) fertilizers on a natural pasture in the Strandzha region leads to an increase in the yield of green mass up to 5.8 times, (*Stoeva, K., V. Vateva, 2007).* For the conditions of the Sakar region, with complete mineral fertilization of a dry field pasture on highly eroded leached cinnamon-forest soil, the yield from the pasture increased 5-8 times *(Dzhingov , Al. et al., 1994).*

Fertilization of eroded terrains has a twofold benefit. On the one hand, the application of fertilizers improves the nutritional status of the soil, creating more favourable conditions for the development of vegetation therein. On the other hand, the introduction of fertilizers improves the water-physical characteristics of the soil which on its part affects the intensity of washing out of the soil. *(Myanushev, E., et al., 2005).*

A study conducted in the period 2005-2008 at the Agricultural Experimental Station – town of Sredets (*Stoeva K. et al., 2010)* demonstrates that on average for the period, the yields of green mass from a perennial grass mixture varied between 2258.1 and 2624.8 kg/daa, and of dry mass - from 778.8 to 861.8 kg/daa when fertilizing with different types and rates of manure.

Most of the territories of Strandzha are characterized as low-mountainous, hilly areas. The hilly areas of Strandzha are formed by dry xerophytic meadows. (*Yancheva, Hr. 2006)* and accordingly *(Stoeva, K et al., 2005),* the pastures in Strandzha are chiefly composed of Bermuda grass / perennial ryegrass (*Cynodon dactylon L. / Lolium perennial L.*). Most of the soils have a humus content of less than 2% and high acidity. The area is one of the driest in Bulgaria. Precipitation is insufficient and as a result of the drought in spring and summer, the grass vegetation ceases its growth and dries up (*Stoeva, K. et al., 2002).* A significant fraction of the pastures also suffer from water erosion, I and II degree.

All this necessitates the combination of appropriate agrotechnical and meliorational measures to improve the general condition in terms of the productivity, quality and botanical composition of the grassland. For sustainable productivity and good quality of the biomass from grass associations, the most reasonable approach is the use of suitable biological fertilizers.

The goal of the study presented herein was to determine the impact of biofertilizer BIOSOL on the productivity and the change of the herbage of natural pastures in the Strandzha Mountains.

MATERIALS AND METHODS

The study was conducted on 4 natural grasslands in the border area of South-Eastern Bulgaria (Strandzha Mountain)

They have been so selected so as to cover the most typical and used natural grasslands in the foothills of the region.

Plot 1 (variant 1) – 240 m altitude, with slope 8°, east exposure.
Plot 2 (variant 2) - 350 m altitude, with slope 6°, south exposure.
Plot 3 (variant 3) - 380 m altitude, with slope 12°, northwest exposure.
Plot 4 (variant 4) - 280 m altitude, with slope 4°, southeast exposure.

Respectively, tests have been carried out on these four plots to monitor the impact of biofertilizer BIOSOL in a dose of 30 kg/da. Fertilization was done manually at the beginning of March and after the first swath. Mowing was done manually during hay maturity phase.

During the period of the study hereof, the following parameters were tracked down: yield of green mass by weighing the mowed fresh mass from the plots; botanical composition of the herbage – it was determined by weight and the percentage of cereals, legumes and miscellaneous grasses was determined; the density of the grass - expressed in pieces/m^2; the grass height - the measurement was made before mowing; chemical composition of the dry matter on the average of the four variants - the percentage content of crude protein, crude fats, crude fiber, minerals and nitrogen-free extract; climatic characteristics and their impact on the productivity of natural pasture grasses.

RESULTS AND DISCUSSION

Meteorological conditions and especially rainfall are the dynamic factors that have a strong influence on the formation of the productivity of natural grasslands.

The meteorological conditions during the three years of the study (2022-2024) differed significantly from each other. Major differences between meteorological conditions were also reported as compared to the standard values for the region.

Regarding rain precipitation (Figure 1), during the vegetative phase, the highest amount of rainfall was measured in 2022 - 595.9 mm, and the least precipitation was marked in 2024 – 439.2mm. In 2023 the reported value of the rainfall in said period was 446.3 mm. Above data reveals that 2023 and 2024 had precipitation below the average value for the region, while precipitation in 2022 was above the average norm for the region - 528.9 mm. Uneven distribution of rainfall during the observed vegetative phase was also noticed.

In terms of temperature (Figure 2), all three years were warm, the average monthly temperatures being significantly higher than the average values for the multi-year period. The table reveals that the average temperatures during the vegetative phase are higher than the average temperature of the region for the multi-year period, that is 9.7°C. More precisely, in 2022 average temperature was 10.12°C, in 2023 - 11.12°C and in 2024 - 12.34°C.

In Figure 3, the botanical composition of the natural grasslands in Strandzha is showcased. The percentage ratio between cereal, leguminous and miscellaneous grasses is given. It can be seen that the pastures have higher content of cereal components - between 31.5% - 47.5% and miscellaneous grasses - between 29.3% - 60.6 %, the increase being due to the lower fraction of leguminous species - between 7.0% - 23.2%. No changes in the botanical composition were observed in the fertilized variants.

The density of the herbage, averaged over the three years (Figure 4) is expressed in pcs/m^2. The number of plants per m^2 in the control plot is smallest in Plot 1 - 1120 pcs/m^2, and largest in Plot 3 - 1420 pcs/m^2. On average for the period, there was no significant difference in the density of the herbage for the fertilized variants.

Plant height averages over the three years of the study is shown in Table 1. The data indicates that before the first cut, the height of the cereal species in the control plot is in the order of 61.2 - 80.5 cm, and of the leguminous species -between 36.8 and 40.0 cm. Before the second cutting, the height of the cereal species is from 36.5 cm to 42.1 cm and from 16.5 cm to 28.5 cm for the leguminous species. The difference in the height of the fertilized variants of the cereal grass compared to the control plot is less than 1 cm. More significant increase in height compared to the control plot was observed in leguminous grasses - from 1.5 cm to 3.2 cm.

The chemical composition of the dry matter on the average for the variants for the three years of the study is shown in Table 2. It shows that in the control plot, the average crude protein content is 6.90%, and crude fiber is 28.90%. Fertilization with biofertilizer BIOSOL leads to an increase in crude protein content up to 8.10% and decrease in the crude fiber to 25.30%. The content of crude fats, minerals and nitrogen-free extracts also increases in the fertilized variants compared to the control plot.

The data on the impact of fertilization with biofertilizer BIOSOL on the yield of green mass by year and on average for the three-year period (2022-2024) are presented in Table 3.

The table clearly demonstrates that 2023 has the highest yields in the control variants, this can be explained by the large amount of precipitation in the spring from March to June (307.6 mm) as well as due to the high temperatures in the winter months and temperatures in the spring close to the average for the region, which led to a rapid growth of the grasses.

The results reveal that the highest yield of green mass on average for the two swaths was recorded in the variant 3 grassland. The control plots values were respectively: for 2022 – 770 kg/da, for 2023 - 775 kg/da. and for 2024 – 765 kg/da. In the fertilized variants these were respectively: 845 kg/da, 827.5kg/d and 820 kg/da, which is most probably due to the higher content of leguminous and cereal grasses and the higher altitude of the pasture.

Variant 1 has the lowest productivity, in all three years with between 460 kg/da and 479 kg/da in the control plot while fertilized variants produced from 482.5 kg/da to 517.5 kg/da green mass.

On average for the three-year period, the amount of yielded green mass in the variants fertilized with biofertilizer BIOSOL increased by 7.0-8.0% compared to the control plots.

Definitely better results were observed on the pastures fertilized with biofertilizer BIOSOL although the weather conditions during the three years of the study differed significantly from each other in terms of both average monthly air temperatures as well as monthly precipitation totals.

CONCLUSIONS

The study of the three-year period allows us to draw the following conclusions:

Fertilization with BIOSOL on natural pastures in the Strandzha region leads to an increase in the yield of green mass by 7.0-8.0%.

After fertilization, there are changes in the height of the grass plants; the content of crude protein in the dry mass is also elevated.

Botanical composition and herbage density were not affected by BIOSOL fertilization.

Fertilization with biofertilizer BIOSOL has a positive effect on the grass characteristics in the natural pastures in the Strandzha Mountain region.

REFERENCES

Boykov, S. 1986. Impact of cleaning and fertilization with mineral fertilizers on natural meadows and pastures. Plant Sciences, 6, 59-63.

Dzhingov, Al., V. Terzieva, L. Lozanova, 1994. Fertilization of natural pasture under conditions of highly eroded cinnamon forest soil in the Sakar region. Soil science, agrochemistry and ecology. 4-6, 143-144.

Godwin, B,J.Thorpe, K.Pivnick and J.Bantle, 1998. Conservation and enhancement of on-farm wildlife habitat and biodiversity. Saskatchevan Research Council Pub.R-1540-5E-98.

Myanushev, E. 2005. Effectiveness of fertilizers in soils with disturbed profile. congratulationsAgrochemistry and ecology (Soil science, agrochemistry and ecology, Bulgaria). (2005). item 40 (2) pp. 45-49.

Owen, O. 1981. Protection of natural resources, Zemizdat, Sofia.

Pavlov, D., 2007. Increasing the Usability of Natural Resources and Natural Grass Associations for Rural Development. Journal Of Mountain Agriculture On The Balkans, v.10, 1, 37-64

Stoeva, K., Vl. Lingorski, Ya. Kozhuharov, 2005. Natural meadows and pastures in Strandzha - basis for biological production of fodder. Balkan Scientific Conference, IZ- Karnobat June 1-3, item II, 574 - 577.

Stoeva, K., V. Vateva, 2007. Influence of mineral fertilization on the productivity and change of the vegetation of natural pastures in Strandzha. Field Crop Studies, v. IV, 2, 369-373

Stoeva, K., V. Vateva, D. Krusheva, 2010. Establishing the productivity of a perennial grass mixture after organomineral fertilization in Strandzha. Journal Of Mountain Agriculture On The Balkans, Troyan, vol. 13, 3, 714–725.

Stoeva, K., Hr. Yancheva, P. Todorova, 2002. Characteristics of natural grass stands in the Strandzhan region. Collection of SUB -St. Zagora, vol. 2, 227-229.

Suthreland, W.J. and Hill, D.A. 1995. Managing habitats for conservation, Cambridge University Press. Cambridge.

Totev, T. 1984. Studies on the improvement and use of natural meadows and pastures in the foothills, mountains and highlands of the Central Stara Planina. Dissertation.

Totev, T., V., Valkov .1988. Responsiveness and efficiency in fertilizing meadow grasses with increasing rates of NPK. Plant Breeding Sciences,7, 49-55.

Yakimova et al., 1977. Improvement and utilization of natural meadows and pastures. Zemizdat, Sofia.

Yancheva, Hr., 2006. Measures for sustainable management and protection of meadows and pastures. www.biodiversity.org.uk

Figure 1. Average monthly precipitation during the vegetation period for the period 2022-2024 years.

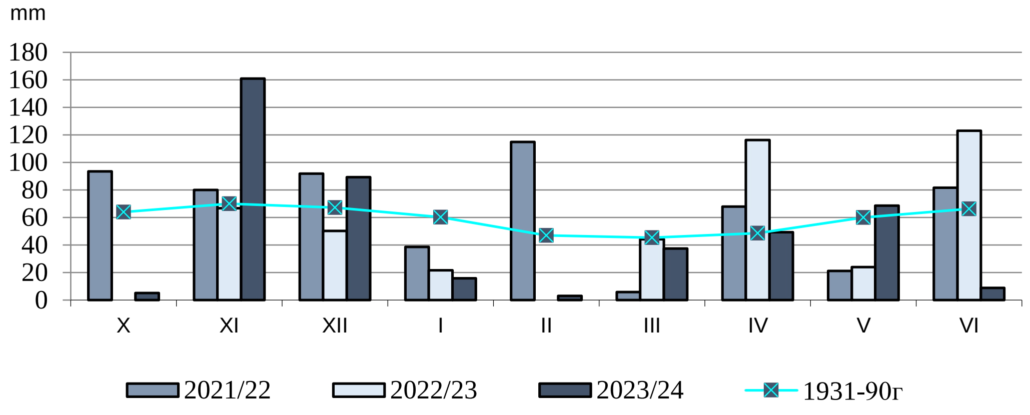


Figure 2. Average monthly temperatures during the vegetation period for the period 2022-2024 years.

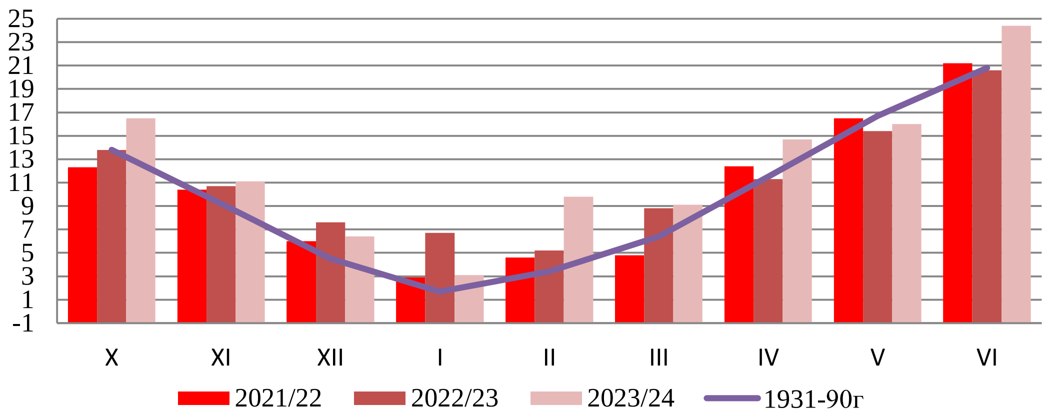


Figure 3. Botanical composition of the herbage averaged over the three years, - %

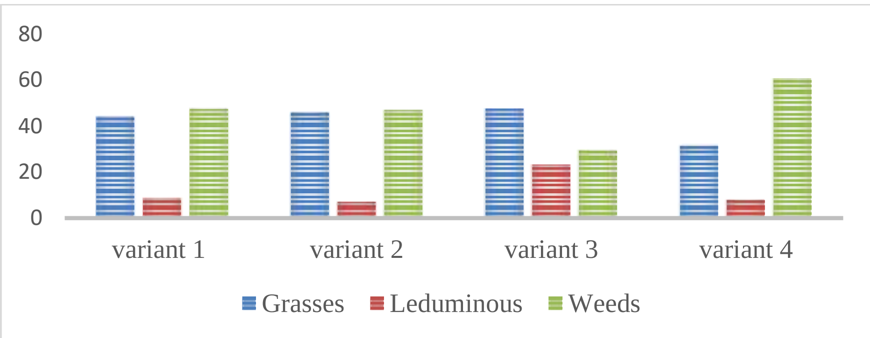


Figure 4. Grass stand density averaged over the three years - pcs/m^2.

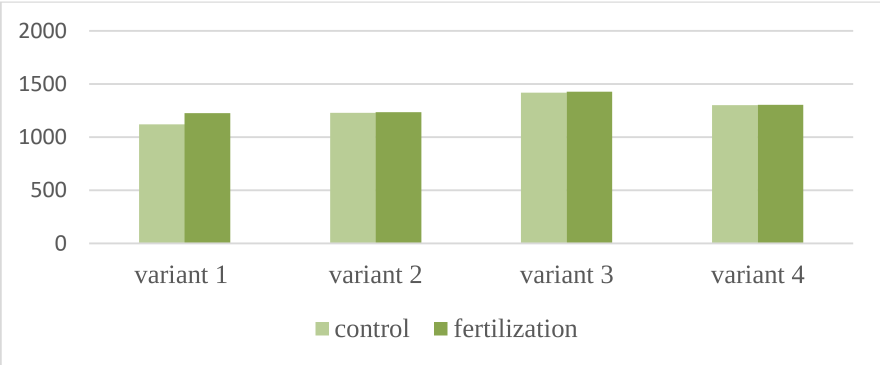


Table 1. Grass height in cm averaged over three years - cm.

Varian	Grass height cm			
	cereal		legumes	
	I grass	II grass	I grass	II grass
Varian 1 control	61.2	38.6	36.5	16.5
Varian 1 fertilization	**61.8**	**41.2**	**36.9**	**18.0**
Varian 2 control	67.4	26.8	38.5	19.0
Varian 2 fertilization	**67.8**	**29.6**	**39.0**	**21.5**
Varian 3 control	80.5	31.5	42.1	28.5
Varian 3 fertilization	**81.2**	**32.4**	**43.5**	**31.0**
Varian 4 control	71.6	40.0	40.0	20.5
Varian 4 fertilization	**72.0**	**40.8**	**41.8**	**23.3**

Table 2. Chemical composition of dry matter averaged for the period - %

Indicators	**Average of the variants**	
	control	fertilization
Crude protein, %	6.90	8.10
Crude fat, %	2.10	2.70
Crude fiber, %	28.90	25.30
Mineral traces, %	6.10	7.00
NFE, %	56.00	56.90

Table 3. Yields on green table by year and average per period - kg/da.

Green mass								
Variants	2022		2023		2024		Mean	
	kg/da	%	kg/da		kg/da		kg/da	%
Varian 1 control	460.0	100.0	479.0	100.0	468.0	100.0	469.0	100.0
Varian 1 fertilization	**482.5**	**105.0**	**517.5**	**108.0**	**513.0**	**110.0**	**504.3**	**107.0**
Varian 2 control	665.0	100.0	695.0	100.0	660.0	100.0	673.3	100.0
Varian 2 fertilization	**705.0**	**106.0**	**767.0**	**110.0**	**715.0**	**108.0**	**729.0**	**108.0**
Varian 3 control	770.0	100.0	775.0	100.0	765.0	100.0	770.0	100.0
Varian 3 fertilization	**845.0**	**110.0**	**827.5**	**107.0**	**820.0**	**107.0**	**830.8**	**108.0**
Varian 4 control	585.0	100.0	630.0	100.0	600.0	100.0	605.0	100.0
Varian 4 fertilization	**630.5**	**107.0**	**670.0**	**106.0**	**648.0**	**108,0**	**649.5**	**107.0**

PHENOTYPIC ANALYSIS OF LEAF SENESCENCE IN *ANTIRRHINUM MAJUS* L.

Esmaeil Chamani[1], Roghayeh Nabipour Sanjbod[1], Younes Pourbeyrami Hir[1]

[1] Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran
Corresponding author e-mail: echamani@uma.ac.ir

ABSTRACT

Leaf senescence represents the final phase of development, culminating in cell death. While leaf senescence poses challenges in the post-harvest lifespan of horticultural plants and the yield of agricultural plants, it plays a crucial role in facilitating the re-mobilization of nutrients within plants. Various factors can influence the regulation of leaf senescence. The natural senescence of the leaf starts and progresses according to a regular schedule. During senescence, morphological and physiological events occur along with intracellular changes. Despite this, these processes have not been thoroughly explored across many species. The snapdragon (*Antirrhinum majus* L) flower serves as a valuable model system that has been extensively studied in terms of genetic traits, flower development, and cellular death. However, the senescence of its leaves has not been investigated. This study delved into the morphological attributes of senescing snapdragon leaves and revealed significant alterations in various indicators as senescence progressed. The results indicated that many indicators changed significantly with the onset and progression of senescence. Notably, there were significant decreases in fresh and dry weight, photosynthetic pigment levels, total soluble protein content, and chloroplast numbers per cell. Conversely, ion leakage and the incidence of dead cells increased with the progression of senescence. Trypan blue staining elucidated that cell death initiation occurred at the leaf tip, progressing to encompass all cells within the leaf blade during the later stages of senescence.

Keywords: Chlorophyll, Chloroplast, Ion leakage, Soluble protein, Trypan blue

INTRODUCTION

Leaf senescence, the final stage of leaf development, is associated with a wide range of morphological, physiological, biochemical, cellular, and molecular changes. Leaf senescence includes changes in leaf color, chlorophyll degradation, breakdown of macromolecules, changes in the intracellular organelles, recycling of nutrients, and the activation of senescence-associated genes (SAGs), and it mainly starts with changes in developmental age (Guo, 2019).

The initial indications of leaf senescence become apparent through changes in leaf color (Zhao et al., 2018). Following a period of rapid expansion and achieving full maturity, a leaf initiates the senescence process, influenced by either the leaf's age or developmental stage or by environmental factors. Typically, the first signs of senescence involve the yellowing of the leaf tip, which gradually spreads inward towards the leaf blade. Eventually, the entire leaf blade takes on hues of yellow, orange, or purple, and the leaf dries up and shrinks, ultimately leading to cell death (Lim et al., 2007; Woo et al., 2018; Zhao et al., 2018).

Senescence is inevitable in all organisms. However, it can be delayed. The delay in senescence can be important in agricultural and horticultural crops in terms of yield and shelf life of crops. It can also impact carbon and nitrogen consumption in plants (Liang et al., 2014). Studies have indicated that senescence syndrome can be evaluated with morphological and physiological changes such as pigment content, nutrient content, ion leakage, and organelle structure. Age-related senescence is different in plant species. At different levels, leaf senescence has been investigated in a few model plants such as *Arabidopsis thaliana* (Buchanan-Wollaston et al., 2005). Therefore, an accurate description of leaf senescence using quantitative methods is very important in other plant species.

For many years, snapdragon (*Antirrhinum majus* L.) has been used as a model system for genetic studies and flower development (Lian et al., 2020; Rabiza-Świder et al., 2020). Recently, its petals have been considered in the evaluation of the programmed cell death (Roghayeh Nabipour Sanjbod et al., 2023a, 2023b). However, there is still no information about the senescence of its leaves. In this study, age-related senescence in snapdragon leaves will be investigated through some basic methods.

MATERIAL AND METHOD

This experiment was conducted at the University of Mohaghegh Ardabili, Ardabil, Iran in 2021 to investigate the leaf senescence of snapdragon. The F_1 seeds of snapdragon (*Antirrhinum majus* cv. Legend White) were purchased from the Takii seed company. The seeds were germinated in Petri dishes (20 °C, LED light, 3000 lux intensity) and sown in plastic pots (length = 18 cm and height = 15 cm) after the selection of coeval germinated seeds. The mixture of pot soil was vermicompost and complete organic fertilizers (3:1). The snapdragon plants were transferred to a greenhouse with standard conditions (15-18 °C, natural daylight) and were grown in December. The irrigation of pots was once every 2 days. Leaf longevity was divided into ten time points and the sampling of leaves was performed at these stages (days 18, 32, 46, 60, 74, 88, 102, 116, 130, 144). The first leaf was analyzed at each time point.

The measurement of fresh and dry weight was performed with a digital Micro Balance (Semi-Micro Analytical Balances GR-200, A&D Company, JAPAN). The samples were dried in an oven (60 °C, 48 h).

For investigation of electrolyte leakage, the leaf segments (squares of 1 × 1 cm) were immersed in deionized water (10 mL) and then placed in a water bath (25 °C, 30 min). After the measurement of initial conductivity (using conductivity meter, WTW InoLab Cond 720 Conductivity Meter, Carl Stuart Limited Company, UK), the samples were incubated in a water bath of 96 °C for 15 min. Then, the total conductivity was calculated and expressed as the percentage of the initial conductivity versus the total conductivity.

The content of chlorophyll and carotenoid was evaluated with the Lichtenthaler method (Lichtenthaler and Buschmann, 2005; Lichtenthaler and Wellburn, 1983). The pigment absorption was determined at three wavelengths (470, 645, and 662 nm) using a spectrophotometer (SP-UV 200, Spectrum Instruments Limited, Australia). The concentration of pigments was calculated using the following equation.

$$C_a\ (\mu g/ml) = 11.24 \times A_{661.6} - 2.04 \times A_{644.8}$$

$$C_b\ (\mu g/ml) = 20.13 \times A_{644.8} - 4.19 \times A_{661.2}$$

$$C_{(x+c)}\ (\mu g/ml) = \frac{(1000 \times A_{470} - 1.90 C_a - 63.14 C_b)}{214}$$

The leaf samples were fixed in 3.5% (v/v) glutaraldehyde for 1 h in the dark for chloroplast number per cell area. The samples were washed with Na2EDTA buffer (0.1 M, pH=9) and kept in this buffer. The samples were then placed in a shaker water bath (60 °) for 2.5 h and stored at 4 °C. The pictures of leaf samples were taken by light microscopy. Image J software was applied for quantification of chloroplast count (Evans et al., 2010).

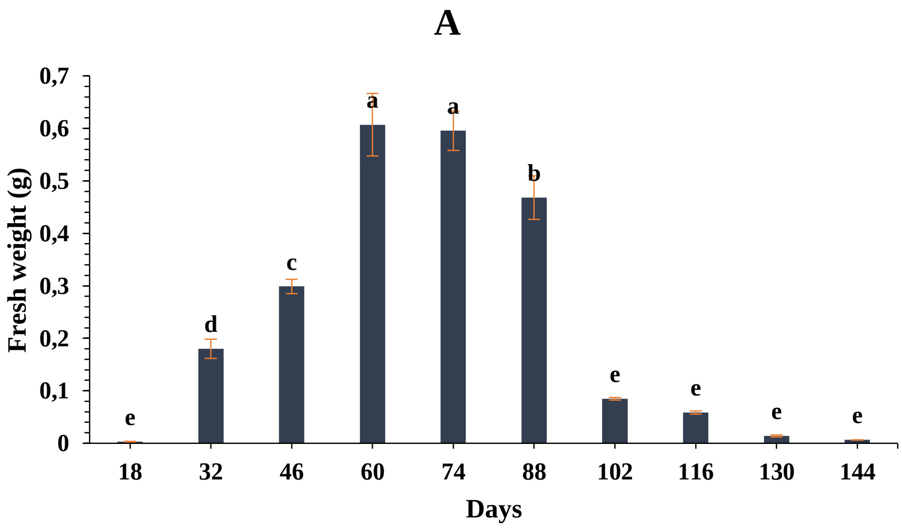
For soluble protein, the leaf tissue (100 mg) was homogenized in phosphate buffer (pH=7) and centrifuged at 10000 rpm (10 min) at 4 °C. The supernatant (100 µL) was separated and mixed with Coomassie solution (5 mL). After vortexing, absorbance was read at 595 nm. BSA standard curve was applied for the calculation of total soluble protein (Roghayeh. Nabipour Sanjbod et al., 2023).

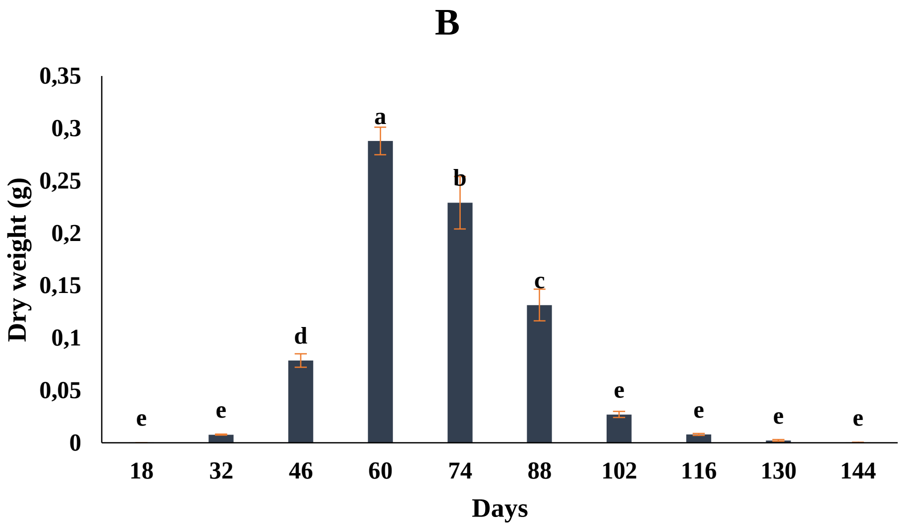
Dead cells of snapdragon leaf were detected with trypan blue staining. For this purpose, the excised leaves were placed in trypan blue staining solution at 96 °C for 1 min. The samples were left at room temperature for 1-24 h. The staining solution was removed and the samples were immersed in chloral hydrate solution for 2–4 h. After that, the chloral hydrate solution was removed and the samples were placed in 70% glycerol. The staining of leaves was recorded by taking pictures (Guo, 2019).

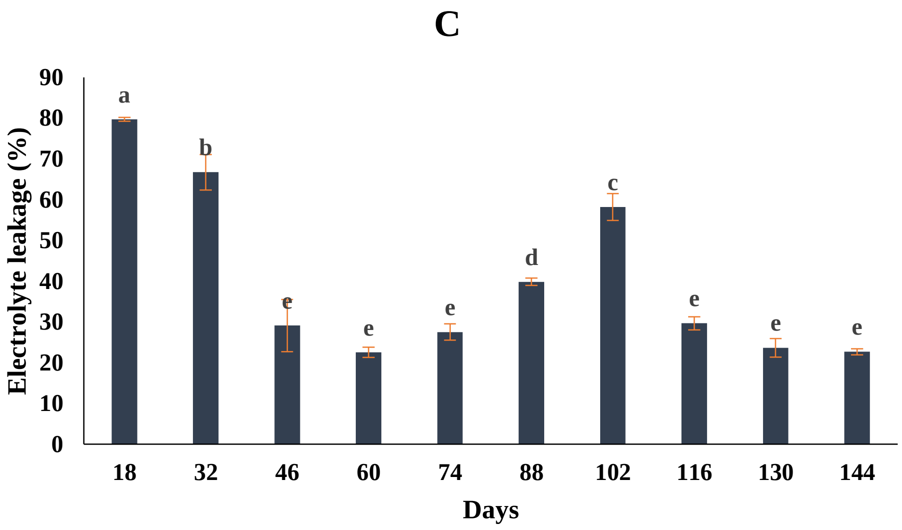
This experiment was conducted as a completely randomized design (CRD) with five replications and analyzed using SPSS software (version 16; SPSS Inc, USA). The means were evaluated with Duncan's multiple-range tests at 5% probability.

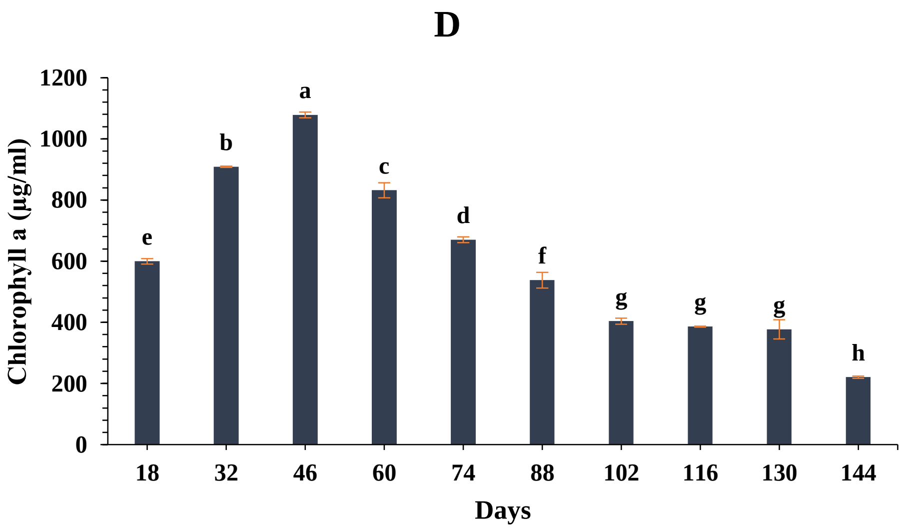
RESULTS AND DISCUSSION

The results of this study showed that with increasing age of the plant, fresh and dry weight increased and reached the maximum value on day 60, then with the onset and progression of senescence, a severe decrease in these parameters was observed, which was visually associated with leaf desiccation (Figure 1A and 1B). Interestingly, the leaves had the highest ion leakage on day 18 in electrolyte leakage. Gradually, with increasing age, ion leakage decreased. On the 88th day, an increase in electrolyte leakage was observed, which coincided with the progress of senescence and the beginning of cell death. After that, ion leakage decreased in the stage of leaf shriveling and desiccation (days 116, 130, and 144) (Figure 1C). The highest content of photosynthetic pigments was observed on the 46th day. After that, the content of chlorophyll a, chlorophyll b, and total carotenoids gradually decreased. (Figure 1D, 1E and 1F) The examination of the total soluble protein content showed that with increasing age and reaching day 88, this parameter increased with a gentle slope, and then with the onset of cell death, cell proteins were broken down and their content significantly decreased (Figure 1G). The highest number of chloroplasts per cell was recorded on the 46th day and their number decreased in the following days (Figure 1H). Trypan blue staining showed that the number of dead cells increased with increasing age. Furthermore, the death of the cells started from the tips and margins of the leaves and gradually the whole leaf cells experienced death (Figure 2).

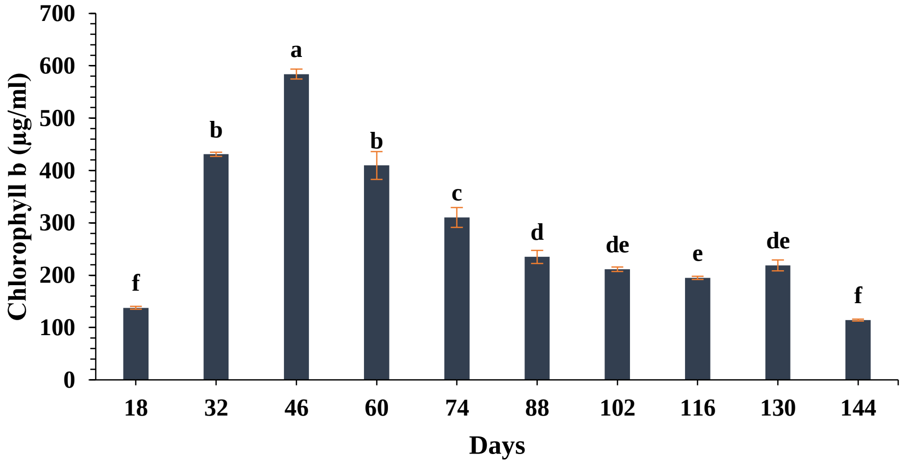



B
Dry weight (g)
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
e
e
d
a
b
c
e
e
e
e
18
32
46
60
74
88
102
116
130
144
Days

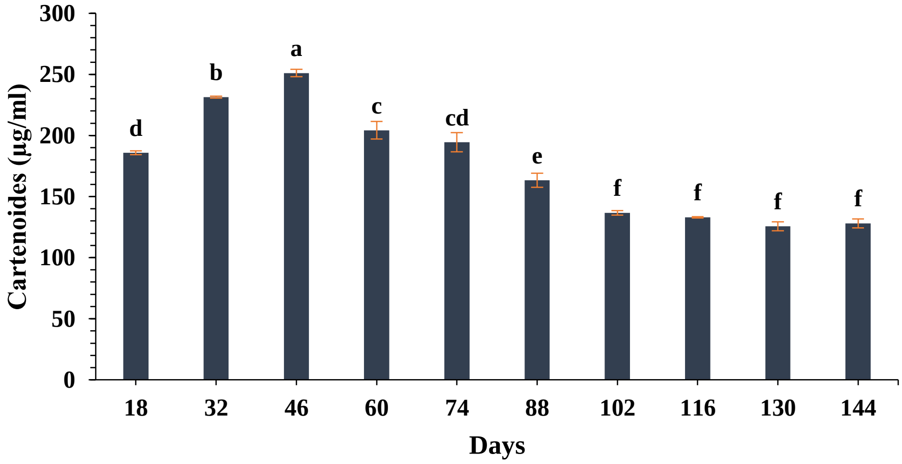

C
Electrolyte leakage (%)
90
80
70
60
50
40
30
20
10
0
a
b
e
e
e
d
c
e
e
e
18
32
46
60
74
88
102
116
130
144
Days


D
Chlorophyll a (µg/ml)
1200
1000
800
600
400
200
0
e
b
a
c
d
f
g
g
g
h
18
32
46
60
74
88
102
116
130
144
Days

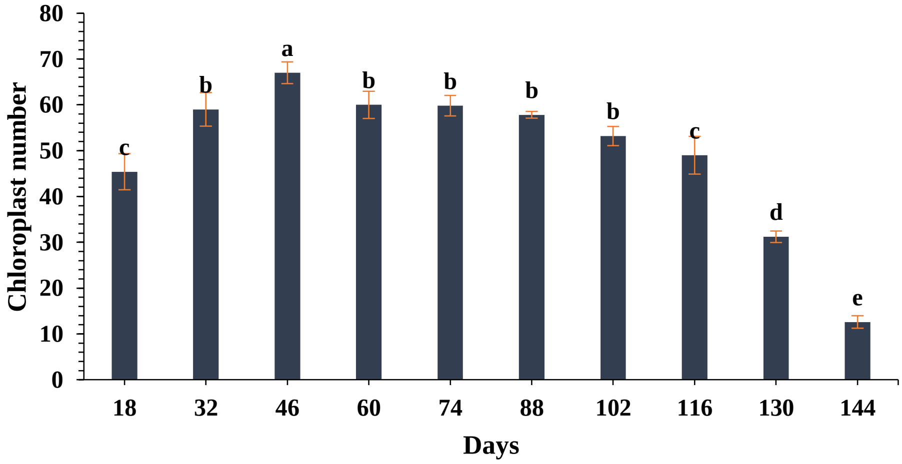
E


Chlorophyll b (µg/ml)
0
100
200
300
400
500
600
700
f
b
a
b
c
d
de
e
de
f
18
32
46
60
74
88
102
116
130
144
Days

F


Cartenoides (µg/ml)
0
50
100
150
200
250
300
d
b
a
c
cd
e
f
f
f
f
18
32
46
60
74
88
102
116
130
144
Days

G


Chloroplast number
0
10
20
30
40
50
60
70
80
c
b
a
b
b
b
b
c
d
e
18
32
46
60
74
88
102
116
130
144
Days

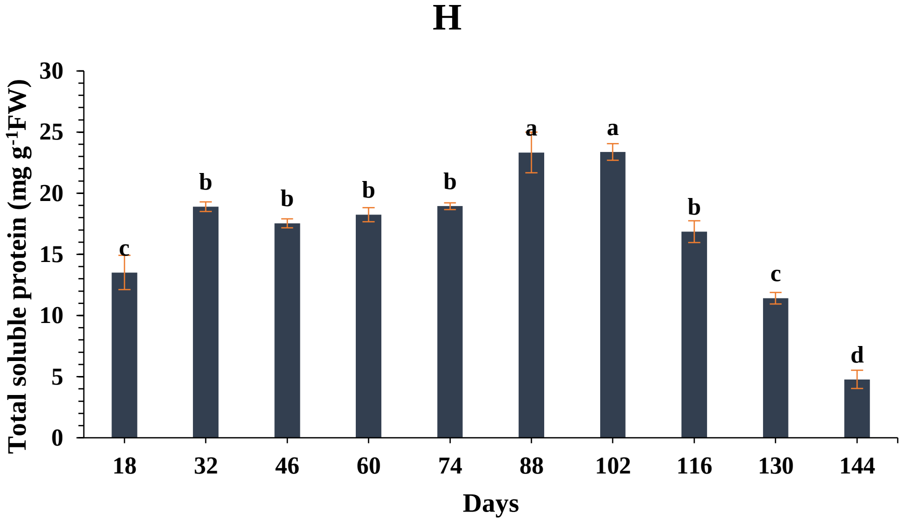


Figure 1. Phenotypic characteristics of snapdragon leaves during senescence. (A): fresh weight, (B): dry weight, (C): electrolyte leakage, (D): chlorophyll a content, (E): chlorophyll b content, (F): carotenoids content, (G): chloroplast number, (H): total soluble protein. Different letters indicate significant differences at p≤ 0.05 (Duncan's test). Error bars represent ±SD, n=5.

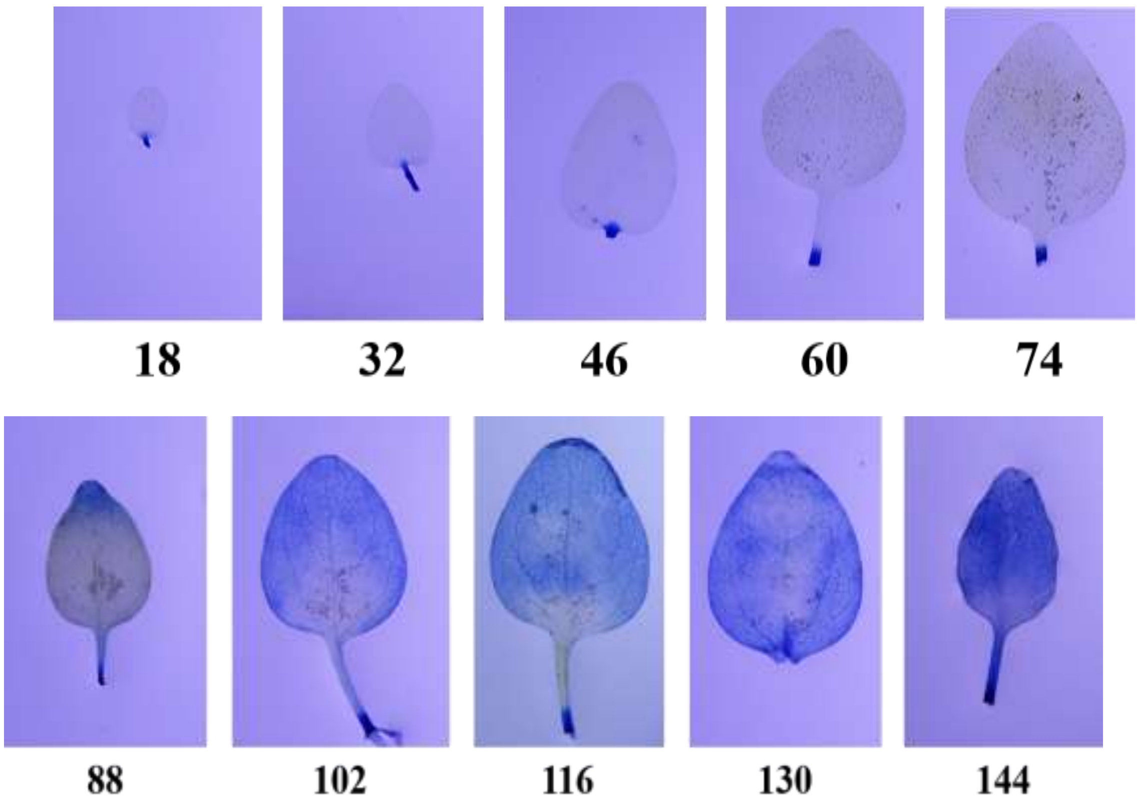


Figure 1. Progression of cell death in snapdragon leaves.

Leaves, as specialized organs for photosynthesis, require considerable energy and nutrients for production; following a productive period, leaves enter senescence where nutrients are redistributed to younger leaves, seeds, or stored for future growth, resembling a recycling program at the organismal level (Quirino et al., 2000).

The senescence of leaves can be discerned through alterations in their appearance. The initial visible indications of leaf senescence involve changes in leaf color. Following growth and maturation, a leaf enters the senescence phase. In typical circumstances, the initial signs of senescence with the yellowing of the leaf tip, progressing gradually towards the leaf blade, eventually resulting in the entire blade, followed by leaf desiccation and shrinkage, culminating in cell death (Guo, 2019) as observed with trypan blue staining.

Chloroplasts are among the primary organelles targeted for degradation at the onset of senescence. Similar to our findings, the loss of chlorophyll is a noticeable occurrence during senescence and is frequently used as a biomarker for the start of senescence. On the other hand, numerous other breakdown processes such as protein, lipid, and nucleic acid degradation also happen (Zhao et al., 2018).

Protein degradation represents a crucial process during leaf senescence, playing a pivotal role in nitrogen recycling. Studies at the transcriptomic level have revealed a significant upregulation of genes related to proteolytic activities (Breeze et al., 2011). Predominantly, serine proteases and CysProt enzymes are prominently associated with leaf senescence, although aspartic, threonine, and metalloproteases have also been identified (Diaz-Mendoza et al., 2016). Plant protease activities have been identified in various cellular compartments such as nuclei, chloroplasts, cytosol, ER, vacuoles, mitochondria, apoplast, cell wall, and specialized vesicles (Diaz-Mendoza et al., 2016). The chloroplast stands as the primary reservoir of mobilizable proteins within the cell, with Rubisco and the chlorophyll-binding light-harvesting proteins of PSII (LHCIIs) being the major proteins (Hörtensteiner and Feller, 2002). Chloroplast proteins account for up to 80% of the total leaf nitrogen and serve as a key nitrogen source during mobilization from senescing leaves. Throughout senescence, chloroplast proteins are believed to undergo degradation through coordinated actions of chloroplast proteases, senescence-associated vacuoles (SAVs), and the ubiquitin/26S proteasome pathway (Diaz-Mendoza et al., 2016).

CONCLUSIONS

The findings revealed that several markers experienced significant alterations as senescence began and developed. Noteworthy was the considerable reduction in both fresh and dry weight, levels of photosynthetic pigments, total soluble protein content, and the number of chloroplasts per cell. In contrast, there was an increase in ion leakage and the prevalence of dead cells as senescence progressed. Analysis with trypan blue staining showed that the initiation of cell death occurred at the tip of the leaf, extending to encompass all cells in the leaf blade in the advanced stages of senescence.

REFERENCES

Breeze, E., Harrison, E., McHattie, S., Hughes, L., Hickman, R., Hill, C., Kiddle, S., Kim, Y.S., Penfold, C.A., Jenkins, D., Zhang, C., Morris, K., Jenner, C., Jackson, S., Thomas, B., Tabrett, A., Legaie, R., Moore, J.D., Wild, D.L., Ott, S., Rand, D., Beynon, J., Denby, K., Mead, A., Buchanan-Wollaston, V., 2011. High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. Plant Cell 23. https://doi.org/10.1105/tpc.111.083345

Buchanan-Wollaston, V., Page, T., Harrison, E., Breeze, E., Pyung, O.L., Hong, G.N., Lin, J.F., Wu, S.H., Swidzinski, J., Ishizaki, K., Leaver, C.J., 2005. Comparative transcriptome analysis reveals significant differences in gene expression and signalling pathways between developmental and dark/starvation-induced senescence in Arabidopsis. Plant J. 42, 567–585. https://doi.org/10.1111/j.1365-313X.2005.02399.x

Diaz-Mendoza, M., Velasco-Arroyo, B., Santamaria, M.E., González-Melendi, P., Martinez, M., Diaz, I., 2016. Plant senescence and proteolysis: Two processes with one destiny. Genet. Mol. Biol. https://doi.org/10.1590/1678-4685-GMB-2016-0015

Evans, I.M., Rus, A.M., Belanger, E.M., Kimoto, M., Brusslan, J.A., 2010. Dismantling of Arabidopsis thaliana mesophyll cell chloroplasts during natural leaf senescence. Plant Biol. 12, 1–12. https://doi.org/10.1111/j.1438-8677.2009.00206.x

Guo, Y., 2019. Plant Senescence: Methods and Protocols. Springer New York.

Hörtensteiner, S., Feller, U., 2002. Nitrogen metabolism and remobilization during senescence, in: Journal of Experimental Botany. https://doi.org/10.1093/jexbot/53.370.927

Lian, Z., Huo, H., Wilson, S.B., Chen, J., 2020. Development of a model mutagenesis system for snapdragon. EDIS 2020, 2–5. https://doi.org/10.32473/edis-ep584-2020

Liang, C., Wang, Y., Zhu, Y., Tang, J., Hu, B., Liu, L., Ou, S., Wu, H., Sun, X., Chu, J., Chu, C., 2014. OsNAP connects abscisic acid and leaf senescence by fine-tuning abscisic acid biosynthesis and directly targeting senescence-associated genes in rice. Proc. Natl. Acad. Sci. U. S. A. 111, 10013–10018. https://doi.org/10.1073/pnas.1321568111

Lichtenthaler, H.K., Buschmann, C., 2005. Chlorophylls and Carotenoids: Measurement And Characterization by UV-VIS Spectroscopy. Handb. Food Anal. Chem. 2–2, 171–178. https://doi.org/10.1002/0471709085.ch21

Lichtenthaler, H.K., Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem. Soc. Trans. https://doi.org/10.1042/bst0110591

Lim, P.O., Kim, H.J., Gil Nam, H., 2007. Leaf senescence. Annu. Rev. Plant Biol. 58, 115–136.

Nabipour Sanjbod, Roghayeh, Chamani, E., Pourbeyrami Hir, Y., Estaji, A., 2023a. Investigation of the cell structure and organelles during autolytic PCD of Antirrhinum majus "Legend White" petals. Protoplasma 260, 419–435. https://doi.org/10.1007/s00709-022-01788-5

Nabipour Sanjbod, Roghayeh, Chamani, E., Pourbeyrami Hir, Y., Estaji, A., 2023b. Autophagic and phytochemical aspects of color changes in white petals of snapdragon flower during development and senescence. Physiol. Mol. Biol. Plants 29, 695–707. https://doi.org/10.1007/s12298-023-01323-7

Nabipour Sanjbod, Roghayeh., Pourbeyrami Hir, Y., Chamani, E., Estaji, A., 2023. LED Lighting Influences Germination, Growth, and Biochemical Indices of Snapdragon. Russ. J. Plant Physiol. 70, 1–9. https://doi.org/10.1134/S1021443723601052

Quirino, B.F., Noh, Y.-S., Himelblau, E., Amasino, R.M., 2000. Molecular aspects of leaf senescence. Trends Plant Sci. 5, 278–282.

Rabiza-Świder, J., Skutnik, E., Jędrzejuk, A., Rochala-Wojciechowska, J., 2020. Nanosilver and sucrose delay the senescence of cut snapdragon flowers. Postharvest Biol. Technol. 165, 111165. https://doi.org/10.1016/j.postharvbio.2020.111165

Woo, H.R., Masclaux-Daubresse, C., Lim, P.O., 2018. Plant senescence: How plants know when and how to die. J. Exp. Bot. 69, 715–718. https://doi.org/10.1093/jxb/ery011

Zhao, L., Xia, Y., Wu, X.-Y.Y., Schippers, J.H.M.M., Jing, H.-C.C., 2018. Phenotypic analysis and molecular markers of leaf senescence. Methods Mol. Biol. 1744, 35–48. https://doi.org/10.1007/978-1-4939-7672-0_3

ASSESSMENT OF ETHYLENE EFFECTS ON POST-HARVEST CHARACTERISTICS OF CUT SNAPDRAGON FLOWERS

Esmaeil Chamani[1], Roghayeh Nabipour Sanjbod[1], Younes Pourbeyrami Hir[1]

[1] *Department of Horticultural Sciences, Faculty of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Ardabil, Iran*
Corresponding author e-mail: echamani@uma.ac.ir

ABSTRACT

Snapdragon (*Antirrhinum majus* L.) flower has unique inflorescence, attractive colors, and shapes, and is known as a cut flower in the world flower market, however, it is considered a sensitive flower to ethylene. Ethylene hormone plays an important role in the senescence of ethylene-sensitive flowers, especially snapdragon flowers, and causes the stem bending of cut flowers. This complication greatly reduces the ornamental value of snapdragon flowers and causes economic damage to producers and consumers. This study investigated different levels of ethylene hormone (0, 1, 10, and 100 µl. L^{-1}) on some growth characteristics and postharvest vase life of snapdragon flowers. The first visible response of snapdragon cut flowers to ethylene treatment was florets dropping whereas the flowers were turgid. The florets dropping was observed in all three levels of ethylene and indicated a significant difference with the control treatment. Ethylene also caused a disturbance in water absorption. Consequently, the relative water content, fresh weight, protein content, and vase life decreased. The curvature angle was also maximum at 100 µl. L^{-1} ethylene.

Keywords: *Antirrhinum majus* L, Curvature angle, Fresh weight, Post-harvest, Senescence

INTRODUCTION

The Snapdragon plant, scientifically known as *Antirrhinum majus* L., is a well-liked decorative plant appreciated for its attractive and aromatic blooms. In the United States, the sales revenue from freshly-cut snapdragons amounted to $12.18 million in 2015, positioning it among the top ten fresh-cut flowers. Challenges arise in the postharvest stage due to the plant's vulnerability to ethylene, which triggers florets' abscission and wilting. This sensitivity contributes to issues like limited vase life, incomplete flower opening, suppression of pigmentation, and stem curvature, impacting snapdragons' overall production and market performance (Kato et al., 2022; Xiang et al., 2020).

Snapdragon petals undergo abscission upon ethylene exposure, suggesting the sensitivity of snapdragon flowers to ethylene. Ethylene is recognized for its pivotal regulatory functions across diverse plant species. The biological activity of ethylene is evident even at very low concentrations ranging from 0.01 to 1.0 parts per million (ppm) (Heffron and Korban, 2022; Kato et al., 2022).

The longevity of cut snapdragon flowers is typically dictated by stem bending, as flowers with bent stems are unsuitable for commercial use. Regrettably, stem bending frequently manifests in snapdragon cut flowers within a brief period of vase life, making it a significant postharvest challenge (Naing et al., 2021). Research using ethylene and ethylene-action inhibitors like silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP) has aimed to determine the involvement of ethylene as a positive regulator in snapdragon stem-bending. However, results varied, with some studies showing no significant impact on bending with STS or 1-MCP, while others reported a blocking effect, suggesting further research is needed to clarify ethylene's role in this mechanism (Çelikel et al., 2010; Naing et al., 2021; Woltering, 2016).

In the present study, we treated snapdragon cut flowers with ethylene and investigated the influence of ethylene on various vase-life characteristics of snapdragon, such as curvature angle and florets abscission.

MATERIAL AND METHOD

Snapdragon F_1 (*Antirrhinum majus* 'Legend White') seeds were germinated in Petri dishes under controlled conditions and placed in a greenhouse with regulated temperature and natural light. The plants were watered every other day until they reached the flowering stage, at which point they were moved to a growth chamber for ethylene treatment. Snapdragon flowers were trimmed to 40 cm and then treated with ethylene at 1, 10, and 100 µl l-1 for 48 h at 22 °C (each treatment with 10 repetitions). The treatment was performed in 38.8 × 39.0 × 38.8 cm glass chambers. The KOH solution (1 M) was placed inside the chamber to reduce CO_2 concentration (Chamani et al., 2005).

The cut stems were weighed on day 0 (before treatment), day 1 (1 day after treatment), day 3 (3 days after treatment), day 5 (5 days after treatment), day 7 (7 days after treatment), day 9 (9 days after treatment), and day 11 (11 days after treatment). The water absorption was also recorded at these time points. The floret's abscission was counted on day 1.

Vase life was determined by recording the number of days from treatment initiation (day 0) until 50% of the flowers in the spike began wilting or drying, indicating the end of their commercial viability. Soluble protein content in the petals was measured using the Bradford assay method to quantify the total protein content (Bradford, 1976) on day 1. Stem curvature in snapdragon cut flowers was measured using a protractor and stated in degrees on day 11. A straight stem was defined 0°.

Statistical analysis of the data was conducted using SPSS V16 software, with significance levels determined through Duncan's multiple range test at a 5% probability.

RESULTS AND DISCUSSION

Gradually, the weight of the shoot in the control increased with a gentle slope and decreased after 5 days. On the other hand, ethylene treatment (1, 10, and 100 µl. L^{-1}) caused a significant decrease in shoot weight 1 day after the treatment. With increasing ethylene concentration, weight loss occurred at a faster rate (Figure 2A). According to the changes in shoot weight, water absorption also revealed a significant increase in the control. Concentrations of 10 µl. L^{-1} and 100 µl. L^{-1} prevented water absorption in flower stems and as a result, the weight of the shoots decreased (Figure 2B). The treatment of cut snapdragon flowers with ethylene (10>100>1 µl. L^{-1}) caused the florets to drop severely. The lowest drop of florets was observed in the control (Figure 2C). As a result, the vase life of untreated flowers was recorded up to 8 days (Figure 2D). Ethylene also caused a significant decrease in total soluble protein content, which indicated the rapid degradation of proteins under ethylene treatment (Figure 2E). Furthermore, the degree of stem curvature observed in E10 and E100 treatments was significantly higher than those of the E1 and control treatments at 11 days after treatment (Figure 2F).

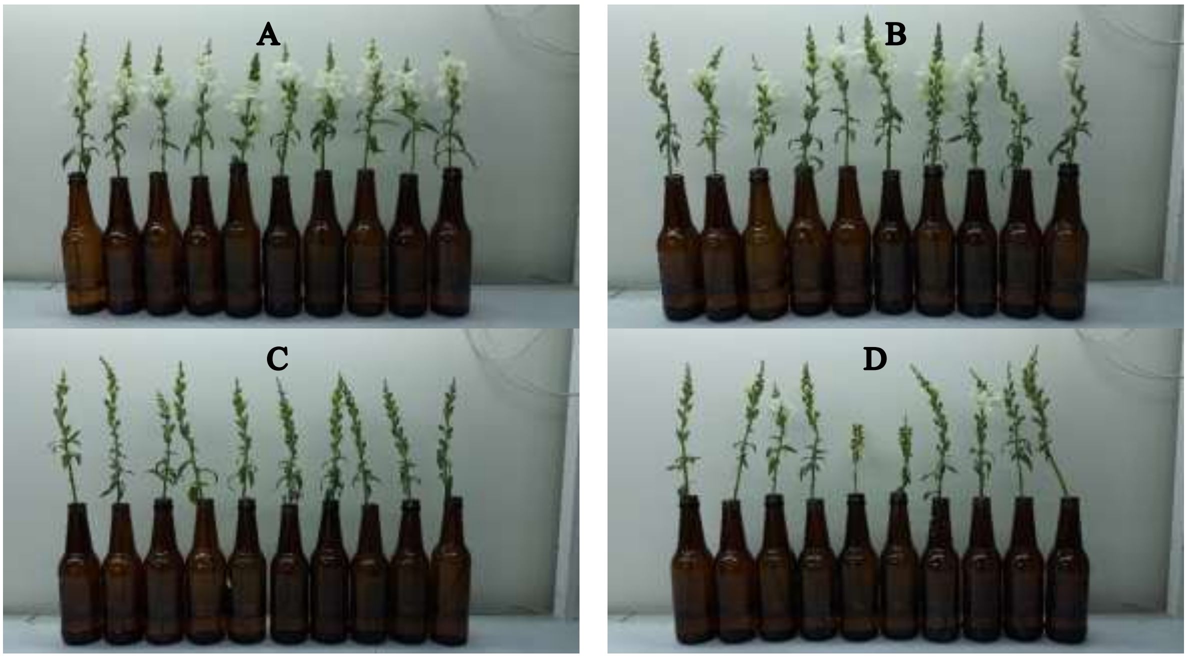
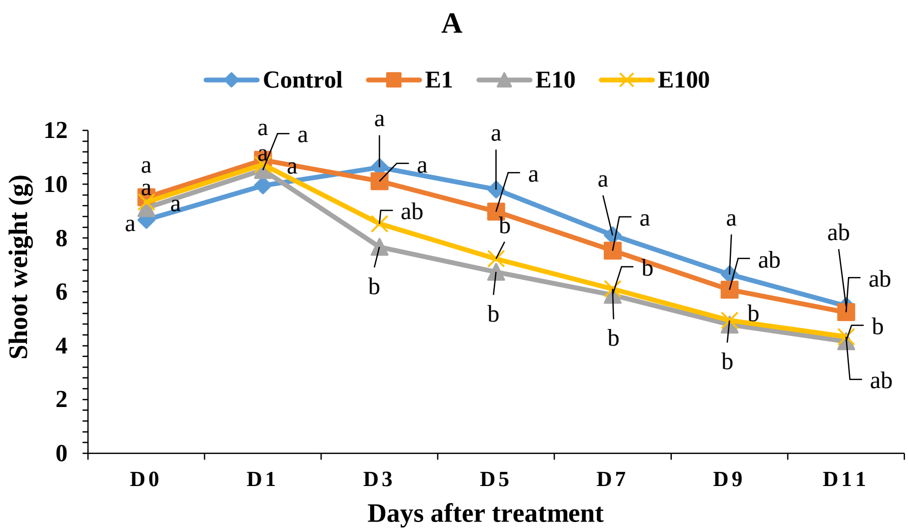


Figure 1. Comparison of the effects of different concentrations of ethylene on postharvest quality of snapdragon cut flowers. (A): control (0 µl. L^{-1}), (B): E1 (1 µl. L^{-1}), (C): E10 (10 µl. L^{-1}), (D): E100 (100 µl. L^{-1}). The photos were taken at day 5.



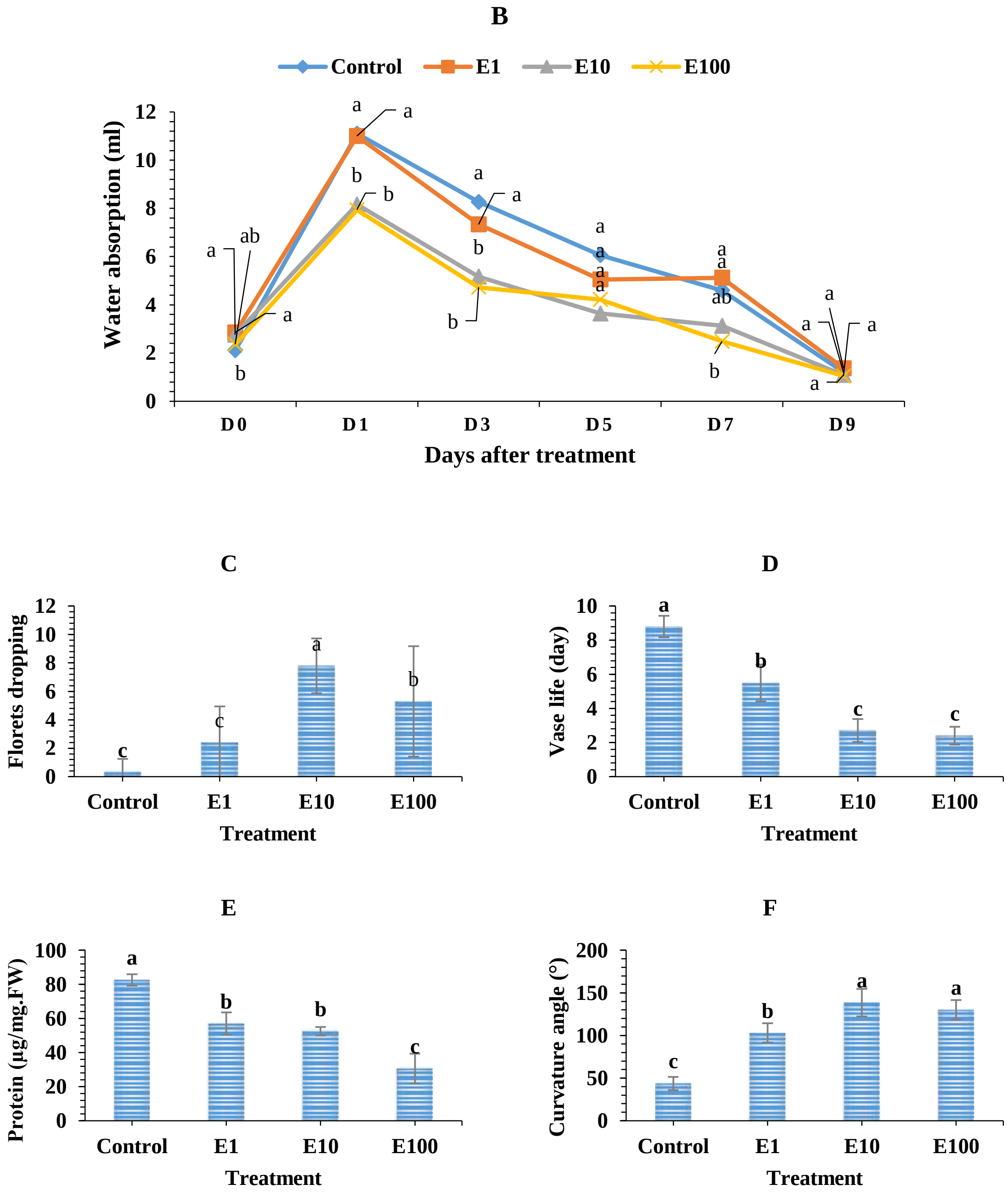


Figure 2. Some post-harvest characteristics of cut snapdragon flowers under ethylene treatment (0 µl. L^{-1}, Control; 1 µl. L^{-1}, E1; 10 µl. L^{-1}, E10; 100 µl. L^{-1}, E100). (A): shoot weight, (B): water absorption, (C): florets dropping, (D): vase life, (E): total soluble protein, (F): curvature angle. Different letters indicate significant differences at $p \leq 0.05$ (Duncan's test). Error bars represent ±SD, n=10.

In this study, we investigated the effect of different concentrations of ethylene on some characteristics of snapdragon flower post-harvest. This study indicated that increasing the concentration of ethylene can strongly increase the angle of curvature and bending of the stem. This incident was accompanied by a decrease in the weight of the stem, a decrease in water absorption, severe dropping of florets, and a lack of soluble protein, which ultimately led to the loss of the useful vase life of the cut flower.

Ethylene can stimulate many degenerative processes in cut flowers (Lukaszewska, 1985). In the present study, different concentrations of ethylene decreased total protein content. Proteins, as vital cellular components, are severely sensitive to adverse environmental conditions. The decrease in total protein is attributed to the involvement of ethylene in the expression of genes related to aging, activation of plant proteases, and proteolytic degradation (Chamani et al., 2007; Eason et al., 2002; Jones et al., 2005).

Many studies have been done on the effect of ethylene regulation on stem bending. However, the results are contradictory and it is not yet clear whether ethylene stimulates stem bending or not (Naing et al., 2021). In a study, the treatment of cut tulip stems with ethephon (ethylene-releasing compound) caused a delay in stem bending (Van Doorn et al., 2011). On the other hand, in some species such as Calendula officinalis and gerbera, ethylene inhibiting compounds have reduced the rate of stem bending, which depends on the structural nature of the stems of different species (Hemati et al., 2019; Lone et al., 2021; Naing et al., 2021).

CONCLUSIONS

This study revealed that the initial observable reaction of snapdragon cut flowers to ethylene exposure was the dropping of florets (in the turgid condition). This phenomenon was evident across all levels of ethylene, highlighting a notable contrast with the control group. Furthermore, ethylene led to a disruption in water uptake, resulting in reductions in relative water content, fresh weight, protein levels, and vase longevity. The highest curvature angle was recorded at an ethylene concentration of 100 µl. L^{-1} and had a significant difference with control.

REFERENCES

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Çelikel, F.G., Cevallos, J.C., Reid, M.S., 2010. Temperature, ethylene and the postharvest performance of cut snapdragons (Antirrhinum majus). Sci. Hortic. (Amsterdam). 125, 429–433. https://doi.org/10.1016/j.scienta.2010.04.005

Chamani, E., Joyce, D.C., Esmaeilpour, B., 2007. Thidiazuron effects on physiochemical characteristics of carnation during pre and postharvest periods. J. Appl. Hortic. 09, 115–117. https://doi.org/10.37855/jah.2007.v09i02.25

Chamani, E., Khalighi, A., Joyce, D.C., Irving, D.E., Zamani, Z.A., Mostofia, Y., Kafi, M., 2005. Ethylene and anti-ethylene treatment effects on cut “First Red” rose. J. Appl. Hortic. 07, 3–7. https://doi.org/10.37855/jah.2005.v07i01.01

Eason, J.R., Ryan, D.J., Pinkney, T.T., O’Donoghue, E.M., 2002. Programmed cell death during flower senescence: isolation and characterization of cysteine proteinases from Sandersonia aurantiaca. Funct. Plant Biol. 29, 1055–1064.

Heffron, L.M., Korban, S.S., 2022. Evaluation of ethylene mutant snapdragon lines for rooting, gravitropism, 1-MCP, ethylene, and vase-life responses. Sci. Hortic. (Amsterdam). 304. https://doi.org/10.1016/j.scienta.2022.111274

Hemati, E., Salehi Salmi, M.S., Daneshvar, M.H., Heidari, M., 2019. The roles of sodium nitroprusside, salicylic acid, and methyl jasmonate as hold solutions on vase life of Gerbera jamesonii ‘Sun Spot.’ Adv. Hortic. Sci. 33, 187–195. https://doi.org/10.13128/ahs-24261

Jones, M.L., Chaffin, G.S., Eason, J.R., Clark, D.G., 2005. Ethylene-sensitivity regulates proteolytic activity and cysteine protease gene expression in petunia corollas. J. Exp. Bot. 56, 2733–2744. https://doi.org/10.1093/jxb/eri266

Kato, M., Kanda, M., Ichimura, K., 2022. Effects of Pulse Treatments with Sucrose, Silver Thiosulfate Complex (STS) and Calcium Chloride on the Vase Life and Soluble Carbohydrate and Aurone Levels in Cut Snapdragon Flowers. Hortic. J. 91, 112–121. https://doi.org/10.2503/hortj.UTD-304

Lone, M.L., Haq, A. ul, Farooq, S., Altaf, F., Tahir, I., 2021. Nitric oxide effectively curtails neck bending and mitigates senescence in isolated flowers of Calendula officinalis L. Physiol. Mol. Biol. Plants 27. https://doi.org/10.1007/s12298-021-00969-5

Lukaszewska, A.J., 1985. The effect of benzyladenine and ethephon on soluble protein content and invertase activity in wilting cut roses cv. Carina, in: III International Symposium on Postharvest Physiology of Ornamentals 181. pp. 87–92.

Naing, A.H., Soe, M.T., Yeum, J.H., Kim, C.K., 2021. Ethylene Acts as a Negative Regulator of the Stem-Bending Mechanism of Different Cut Snapdragon Cultivars. Front. Plant Sci. 12. https://doi.org/10.3389/fpls.2021.745038

Van Doorn, W.G., Kirasak, K., Sonong, A., Srihiran, Y., Van Lent, J.W.M., Ketsa, S., 2011. Do plastids in Dendrobium cv. Lucky Duan petals function similar to autophagosomes and autolysosomes? Autophagy 7, 584–597. https://doi.org/10.4161/auto.7.6.15099

Woltering, E.J., 2016. Flower Senescence. Encycl. Appl. Plant Sci. 1, 292–299. https://doi.org/10.1016/B978-0-12-394807-6.00010-1

Xiang, D., Nguyen, C.D., Felter, L., Clark, D., Huo, H., 2020. The effects of preharvest LED light, melatonin and AVG treatments on the quality of postharvest snapdragon and vase life. J. Floric. Landscaping 14–19. https://doi.org/10.25081/jfcls.2020.v6.6236

DEVELOPMENT OF AN INNOVATIVE APPLICATION FOR THE PRESENCE OF *Cydalima perspectalis* in BOXWOOD AREAS OF TURKIYE BY USING GOOGLE EARTH ENGINE

Sule YAMAN[1], *Mustafa YAMAN*[2]

[1] *Karadeniz Technical University, Engineering Faculty, Department of Geomatics Engineering, Trabzon, Turkiye*
[2] *Bolu Abant İzzet Baysal University, Faculty of Arts and Science, Department of Biology, Bolu, Turkiye*
Corresponding author e-mail: suleyaman98@hotmail.com

ABSTRACT

Boxwood (*Buxus* spp.) is used as a forest tree and ornamental plant. It is distributed all over the world with 105 different species except Antarctica and is evaluated as a raw material source in various areas. However, boxwood (*Buxus* spp.), which provides both ecological and economic contributions, is unfortunately under the threat of an invasive pest, *Cydalima perspectalis* (boxwood moth). *C. perspectalis* has rapidly increased its presence in our country every year. The damage caused by the drying up of boxwoods by pests leads to serious economic and ecological losses. Therefore, developing methods to control this pest and taking early measures is of critical importance. In this study, it was aimed to implement a map application consisting of *C. perspectalis* information in boxwood locations in order to perform early detection of *C. perspectalis* in Turkiye and to take the necessary precautions. In this direction, field studies were carried out and coordinate information of boxwood locations in Turkiye was obtained. At the same time, the presence of *C. perspectalis* on boxwoods was confirmed with field studies. After the field studies, a data set was created with coordinate, date, altitude and *C. perspectalis* presence/absence information. The created data set was transferred to the Google Earth Engine (GEE) platform and visualized. In addition to the data set, an NDVI vegetation index graph was created using Sentinel-2A satellite images belonging to the date of the field studies. An application was developed with the codes written on the GEE platform and the date, altitude, *C. perspectalis* presence/absence and NDVI (Normalized Difference Vegetation Index) graph information of the clicked boxwood locations were shown on the panel. This study provides the first preliminary view of the presence of *C. perspectalis* in the same locations and dates in future periods.

Keywords: *Cydalima perspectalis*, Google Earth Engine, Boxwood

INTRODUCTION

Boxwood species naturally distribute in Turkiye. These plants, which are generally found in natural populations in forested areas, are also widely used as landscape plants in parks and gardens (Sarı and Çelikel 2019).

However, despite its widespread use and distribution, boxwood is facing the danger of extinction in our country, as well as all over the world, due to reasons such as harmful insects, diseases, and climate change. In particular, the invasive boxwood moth (*Cydalima perspectalis*) threatens this plant (Billen 2007, Van der Straten and Muus 2010).

Studies have shown that *C. perspectalis* produces 2, 3, 5 generations per year and that this situation varies according to climate and geographical conditions (Chen, 2005; Öztürk et al., 2016; Toper Kaygın and Taşdeler 2019; Yıldız 2021). When the ecological and morphological characteristics of *C. perspectalis* are examined, it is observed that it spreads very quickly due to its ability to feed on different hosts, its high flight speed ability and its ability to produce multiple generations (Matosevic, 2013).

A number of precautions need to be taken to prevent spread, control pests and ensure early detection.

Reviewing the literature on this subject, it is seen that the method of pest control is mostly biological control (Gugea and Vîrteiu 2017; Plant et al., 2019; Salioğlu 2020).

Recently, it has been observed that Geographic Information Systems (GIS) and remote sensing (RS) techniques and technologies, which have been widely used in pest control, early detection, monitoring, mapping, analysis and interpretation of damage caused by pests, are not used sufficiently either in the world or in Turkiye.

For these reasons, the aim of this study was to develop a map application consisting of *C. perspectalis* information in boxwood locations in order to enable early detection of the boxwood moth (*C. perspectalis*) in Turkiye and to take the necessary precautions.

For this purpose, cloud-based Google Earth Engine (GEE) platform and NDVI (Normalized Difference Vegetation Index) vegetation index, which are among UA and GIS technologies, were used.

MATERIAL AND METHOD

Turkiye was chosen as the study area due to its rich vegetation, suitable topography, climate and most importantly, its occupation by *C.perspectalis*. Coordinate information was obtained from 45 locations where boxwood is found in locations of Turkiye through field studies (Figure 1).

Figure 1. Study area and boxwood locations

During the field studies, the presence of *C. perspectalis* on the boxwoods was simultaneously observed and recorded along with the field dates and altitude. A database was created for the study in line with this information. The created database is given in Table 1.

Table 1. Database created through field studies

Province	District	Date	Latitude	Longitude	Altitude	*Cydalima perspectalis*
Adana	Aladağ	11.02.2023	37,472725	35,415433	841	No
Adana	Feke	11.02.2023	37,875833	35,846389	760	No
Adana	Kozan	03.07.2023	37,523192	35,887592	418	Yes
Ankara	Bilkent	20.05.2022	39,878894	32,763065	987	Yes
Antalya	Kumluca	17.11.2022	36,674724	30,56324	11	No
Antalya	Adrasan	17.11.2022	36,3181	30,468091	172	No
Artvin	Hatila Valley	01.07.2022	41,1917595	41,7465325	487	Yes
Bartın	Ulus	18.05.2022	41,700119	32,787793	842	No
Bilecik	Abadiye	01.05.2024	40,162467	29,736998	611	Yes
Bolu	Merkez	23.05.2022	40,730776	31,600048	728	No
Bolu	Merkez	07.06.2022	40,727694	31,589538	727	Yes
Bolu	Göynük	25.07.2023	40,453774	30,782739	920	No
Bursa	Çiviliçam	20.11.2022	39,913512	28,695068	760	No
Düzce	Merkez	07.08.2022	40,839721	31,15567	149	Yes
Giresun	Merkez	20.07.2023	40,908196	38,358613	11	Yes
Giresun	Dereli	23.07.2023	40,634912	38,384502	847	No
Giresun	Dereli	23.07.2023	40,695057	38,439748	516	No
İstanbul	Subaşı	12.06.2022	41,227502	28,44964	75	No
İstanbul	Şile	27.10.2022	41,070757	29,796066	32	Yes
İzmir	Aliağa	11.02.2023	38,802361	26,97705	16	No
Karabük	Keltepe	17.05.2022	41,096242	32,526848	803	Yes
Kastamonu	Azdavay	19.08.2022	41,596535	33,200209	1238	Yes
Kastamonu	Pınarbaşı	17.05.2022	41,6005434	33,1303023	797	Yes
Kastamonu	Pınarbaşı	19.08.2022	41,603045	33,111549	666	Yes
Kastamonu	Kurtgirmez	19.08.2022	41,590214	33,2077	1171	Yes
Manisa	Şehzadeler	12.07.2023	38,638218	27,441271	25	No
Muğla	Marmaris	18.11.2022	36,827249	28,243102	6	No
Ordu	Altınordu	10.02.2023	40,974944	37,96825	7	Yes
Rize	Zilkale	22.07.2023	40,907621	40,948851	996	Yes
Rize	Zilkale/Meydanköyü	22.07.2023	40,902112	40,946254	1020	Yes
Rize	Çamlıhemşin	11.02.2023	40,897286	40,942511	1056	No
Sakarya	Taraklı	29.10.2022	40,489545	30,555345	1087	No
Sakarya	Taraklı/Uğurlu village	28.10.2022	40,491776	30,559678	1124	No
Sakarya	Taraklı/Kemaller Village	28.10.2022	40,501315	30,586408	1162	No
Samsun	Terme	20.07.2023	41,170052	37,056641	5	Yes
Samsun	Fatsa-Kumru	20.07.2023	40,863116	37,278007	708	No
Sinop	Durağan	02.09.2022	41,36246	34,996704	538	Yes
Trabzon	Merkez	09.02.2023	41,005602	39,73099	32	No
Trabzon	Pelitli	20.07.2023	40,990173	39,788589	66	Yes
Trabzon	Sürmene-Yeniköy	21.07.2023	40,775848	40,052544	605	No
Trabzon	Hayrat	21.07.2023	40,791757	40,381897	992	No
Trabzon	Araklı	23.07.2023	40,724553	40,015572	1251	No
Trabzon	Maçka	29.06.2022	40,7994756	39,7087916	807	No
Trabzon	Arsin	23.07.2023	40,709464	39,825475	1063	No
Zonguldak	Yenice	19.08.2022	41,1981116	32,3667461	170	No

The database prepared via Excell was converted to be suitable for working on the GEE platform. The converted database was loaded onto the platform with the steps “Assets-> New-> CSV file (.csv)”. Coding was done in accordance with the purpose of the study using the coding panel provided by GEE to the users (Figure 2). With the coding, the locations were marked on the map, NDVI time series graphs were produced with Sentinel-2A satellite data with a resolution of 10 m based on the months of September, October, November, February, May, June, July and August, when the pest of the locations reproduced in Turkiye, and also the date, altitude, *C. perspectalis* presence/absence information and NDVI graphs were shown on the panel for each clicked location. Thanks to this coding, the panel changes for each clicked location and the necessary information for that location is shown.

Figure 2. Coding panel

RESULTS AND DISCUSSION

As a result of the study, an application was developed that provides preliminary views and information to users by visualizing the database generated and created through field studies on the GEE platform (Figure 3).

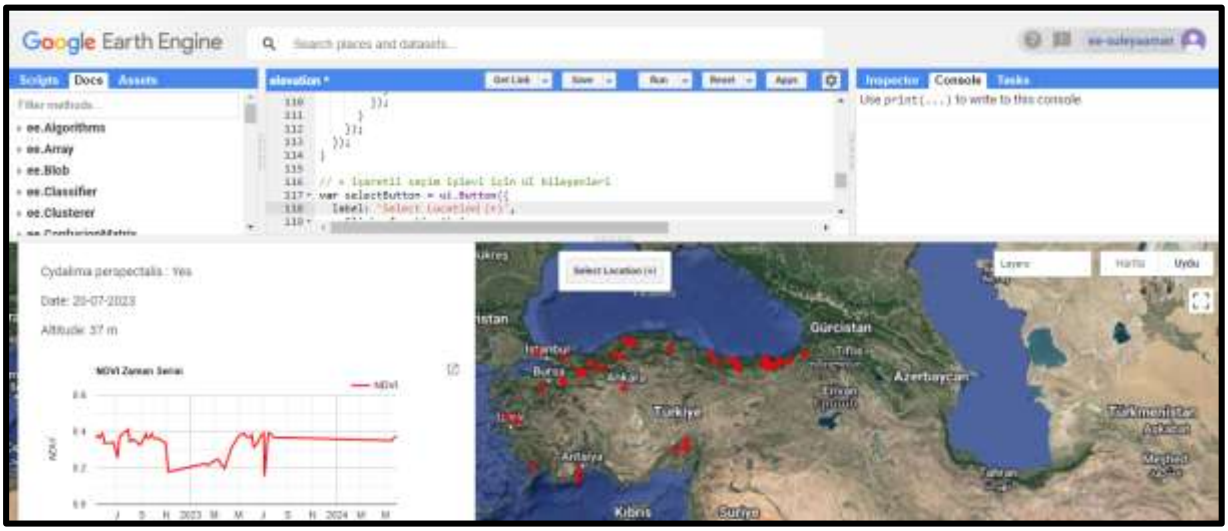


Figure 3. Application developed on Google Earth Engine platform

Sample representations of some of the results obtained with the application are given in Figure 4.

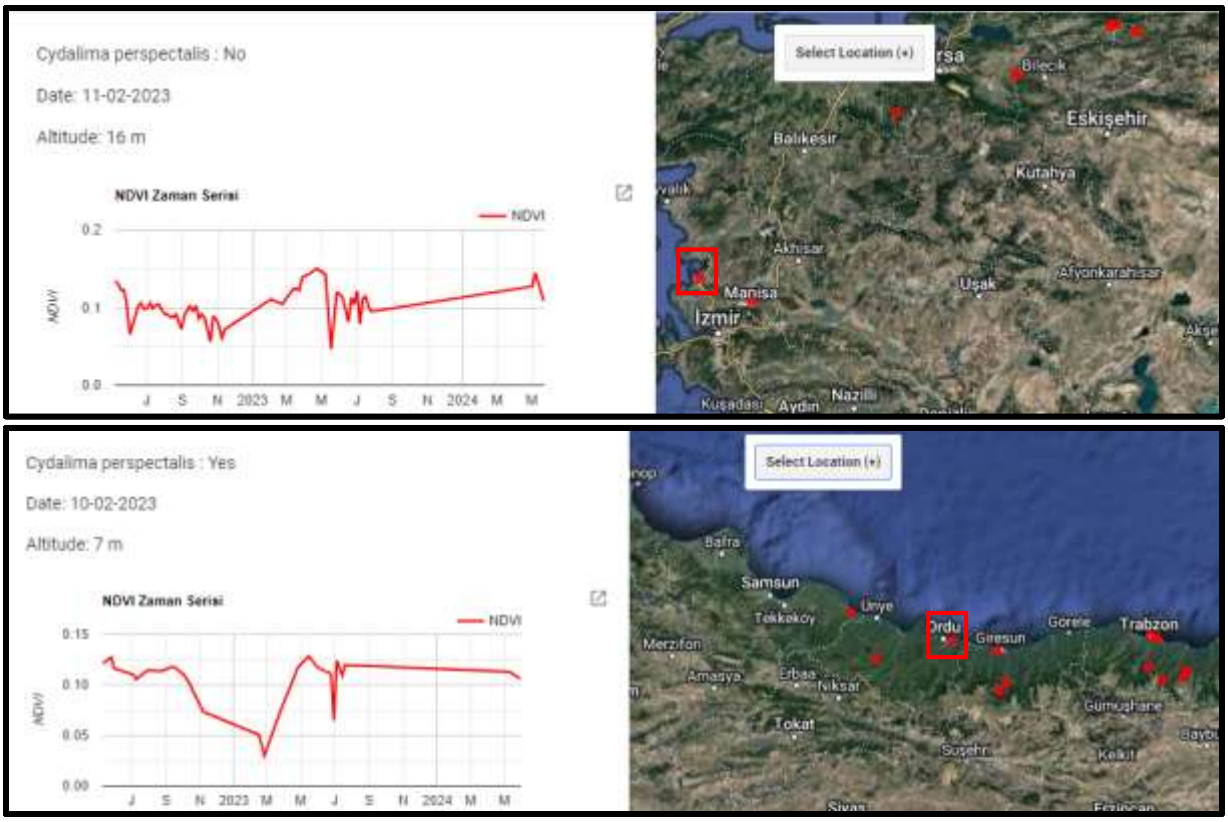


Figure 4. Some sample demonstrations of application results

With the application, it was aimed to monitor the damage caused by the pest with NDVI time series graphs. The use of plant indices such as NDVI in the detection of pests is an approach that facilitates the monitoring of the pest and the determination of the damage it causes. NDVI is a vegetation index that takes a value between -1 and +1, which expresses plant vitality. In this context, while the NDVI value of healthy plants approaches +1, it approaches -1 in cases where there are no signs of life or the vegetation is not alive. When the results of the NDVI time series graphs obtained in this context are examined in detail, it is observed that although the NDVI values do not fall into minus for all months, they vary between 0.1 and 0.3 and make irregular fluctuations. The reason for this situation is that boxwood, which is usually in the form of a bush, cannot be clearly separated from different plant species nearby. Another reason is that the highest resolution satellite data offered for free use has a maximum resolution of 10 m. Increasing the resolution of the satellite data used will provide a high-level solution to this situation. Another reason is that NDVI time series graphics cover a very long time and that events such as rain, snow, clouds, fog, etc. experienced during this time period cannot be completely eliminated even though masking is applied, which is why sufficient elimination cannot be made in the images. For these reasons, it has been concluded that NDVI results should be improved with different techniques.

The application, which works smoothly and beautifully, has been implemented for 45 locations. Increasing the number of locations will increase the efficiency of the study and will be a useful approach to create a complete *C.perspectalis* and boxwood distribution map of Turkiye.

When the altitude criteria and dates were evaluated, *C. perspectalis* was observed at 5 m in July and 1238 m in August, but not at 32 m and 1056 m in February. This shows that altitude is not the only criterion for the presence of *C. perspectalis*, and that criteria such as temperature, precipitation, humidity, etc. in the current month are also important.

CONCLUSIONS

During the application, some limitations were encountered due to reasons such as the inadequacy of current technologies and the physiological structure of plant species. Despite this, the application developed with this study works perfectly and flawlessly.

This study provided the first preliminary view of the presence of *Cydalima perspectalis* in the same locations and dates in the future periods and made it possible to detect and combat the pest early. Since this study has no example in the world or in Turkiye, it has a great place in terms of being a hope and an example for future studies.

It is aimed to carry the study further with the improvement of current technologies and the contributions of researchers. In this direction, it is expected that university-public cooperation will increase and the database will be kept up to date.

ACKNOWLEDGMENT

The study was financially supported as a research project by the Scientific and Technological Research Council of Turkiye (221O401).

REFERENCES

Billen W (2007) Diaphania perspectalis (Lepidoptera: Pyrallidae)-a new moth in Europe. Mitteilungen der Entomologischen Gesellschaft Basel, 57(2/4), 135-137.

Chen, H. L., Gao, Z. G., Zhou, J. M., & Chen, H. M. (2005). Bionomics of the box tree pyralis, Diaphania perspectalis (Walker). Ji-Angxi Plant Prot, 28, 1–4.

Gugea I., Vîrteiu AM. (2017). Remains Cydalima perspectalis (Lepidoptera: Crambidae) an invasive species dangerous for landscaping in western Romania?, Research Journal of Agricultural Science, 49 (4): 135-141.

Matosevic, D. (2013). Box Tree Moth (Cydalima perspectalis, Lepidoptera; Crambidae), New Invasive Insect Pest in Croatia. South-east Eur for 4 (2): 89-94.

Öztürk, N., Akbulut, S., & Yüksel, B. (2016). Düzce İçin Yeni Bir Zararlı Cydalima perspectalis (Walker, 1859) (Lepidoptera: Crambidae). Düzce Üniversitesi Orman Fakültesi Ormancılık Dergisi, 12(1), 112-121.

Plant CW, Poole C, Salisbury A, Bird S (2019) The Box-tree Moth Cydalima perspectalis (Walker, 1859) in Britain: an overview of its spread and current status. Entomologist's Record and Journal of Yesiation, 131, 122-147.

Salioğlu Ş (2020) Artvin ili şimşir alanlarında zarar yapan Cydalima perspectalis (Walker, 1859) (şimşir kelebeği)'in morfolojisi, biyolojisi, zararı ve mücadele olanaklarının araştırılması. Yüksek lisans tezi. Artvin Çoruh Üniversitesi, Lisansüstü Eğitim Enstitüsü, Artvin.

Sarı Ö, Çelikel FG. (2019). Türkiye'nin Şimşirleri (Buxus sempervirens ve Buxus balearica) ve Mevcut Tehditler. I. International Ornamental Plants Congress – VII.Süs Bitkileri Kongresi, 9-11 Ekim 2019, Bursa, S.383-393.

Toper Kaygın, A. & Taşdeler, C. (2019). Cydalima perspectalis (Walker) (Lepidoptera: Crambidae, Spilomelinae)'in Türkiye'de Coğrafi Yayılışı, Yaşam Döngüsü Ve Zararı . Bartın Orman Fakültesi Dergisi , 21 (3) , 833-847 . DOI: 10.24011/barofd.578294.

Van der Straten M J, Muus TS (2010) The box tree pyralid (Glyphodes perspectalis (Walker 1859), Lepidoptera: Crambidae); an invasive alien moth ruining box trees. In Proceedings of the Netherlands Entomological Society Meeting, 21, 107-111.

Yıldız Y. (2021). Damage, Biology and Control Of Box Tree Moth Cydalima perspectalis In Bartın. 1st International Workshop on Boxwood ., 25-27 October, Rize, Turkey.

ABUNDANCE AND BIOMETRIC CHARACTERISTICS OF Rhopılema nomadica (GALIL, SPANIER & FERGUSON, 1990) PASSING FROM GULF OF ANTALYA TOWARDS THE WEST

Mehmet GÖKOĞLU[1] *Mesut YILMAZ*[1] *Alper YILDIZ*[1]
Akdeniz University, Fisheries Faculty, Antalya, Türkiye
Corresponding author e-mail: gokoglu@akdeniz.edu.tr

ABSTRACT

Rhopilema. Nomadica (Galil, Spanier & Ferguson, 1990) was initially observed in the Mediterranean Sea in the 1970s near the shores of Israel. It is classified as a Lessepsian jellyfish species. From the early 1980s onwards, these jellyfish have begun to gather in significant numbers near the shores of Israel, resulting in negative impacts on fisheries, tourism, the environment, thermal power plants, and human health. *R. nomadica* was not only found along the shores of Israel, but its presence also expanded to the coasts of Egypt, Palestine, Lebanon, Syria, Turkey, Greece, and Italy due to prevailing winds and current systems. The first detection on the Turkish coastline was on the eastern shores of the Göksu River between 1980 and 1982. *R. nomadica*, known for its crowd populations along the coasts of Israel, Lebanon, Syria, and the Gulf of Iskenderun, is shifting westward from the Gulf of Antalya coast between March and May. This movement is attributed to factors like global warming, eutrophication, overfishing, and a decline in its predators. Some of these jellyfish also enter the shallows of the gulf and cause concerns. In 2024, the first migration passings in the Gulf of Antalya started in late January and were completed in late April. The population density in the Gulf increased by 1 individual per 10 m^2 between March and April. In two samplings carried out between February and April, a total of 44 individuals were taken and transported to the research laboratory of Akdeniz University Fisheries Faculty. The average weight of the *R. nomadica* species migrating through Antalya Bay was 3780.11±304.85g, with a mantle diameter (mean±SE) of 37.25±1.40cm.

Keywords: *Rhopilema nomadica*, Biometric Characetistics, Abundance

INTRODUCTION

Rhopilema nomadica Galil Spanier & Ferguson, 1990, a Lessepsian jellyfish, was first recorded in the Mediterranean in the 1970s off the coast of Israel (Galil et al. 1990). It is reported that this planktotrophic jellyfish regularly forms significant swarms along the Levantine coast, depleting plankton resources in this ultra-oligotrophic part of the Mediterranean, and causing damage to tourism, fisheries and coastal facilities (Galil 2007). *R. nomadica* is considered one of the “100 Worst Invasive Species” in Europe. Its rapid proliferation and spread in the Eastern Mediterranean, reaching high densities, confirms this view (Deidun et al 2011).

On the Mediterranean coast of Türkiye, *R. nomadica* was first recorded in 1995 off the coast of Mersin (Kideys and Gücü 1995) and subsequently in İskenderun Bay (Avsar et al. 1996). Since then, large jellyfish aggregations have been observed at certain locations along the Mediterranean coast of Türkiye, particularly during the summer months. It has been reported that the high densities of *R. nomadica* on the eastern Mediterranean coast of Türkiye may be due to the high productivity and pollution of the sea in this region (Kideys and Gücü 1995). These researchers reported that in August 1995, many swimmers on the coast of Mersin had to undergo medical treatment due to contact with jellyfish. They also reported a decrease in the local fishermen's catches with gillnet fishing in that region and that jellyfish caught in their nets were a major problem. In addition, fish farms in İskenderun also experienced difficulties when they wanted to collect fish from cages due to the mass jellyfish population.

Figure 1. The presumed distribution route of *Rhopilema nomadica* in the Mediterranean to date (Deidun et al 2011).

The enormous distribution of *Rhopilema nomadica* in the eastern Mediterranean basin is promoted by its unique life cycle, which includes polyp asexual reproduction and resting periods through podocyst formation, and polydisc strobilation, which results in the release of multiple ephyrae from each polyp. *R. nomadica*, which is extremely abundant in the eastern Mediterranean basin, is drifted westward each year by currents and winds, following the path shown in Figure 1. Some of these jellyfish, which also migrate from the open sea of the Gulf of Antalya, are causing concerns by entering the gulf. The jellyfish, which started migrating in the Gulf of Antalya in January 2024, completed this migration in late April.

Due to the warmer winter and long northern winds, an extreme density of jellyfish was observed in the gulf this year. This study aimed to determine the average stock density, weight, and mantle diameter of jellyfish in the Gulf of Antalya.

MATERIAL AND METHOD

This research was carried out in the Falez region (36°52'16.51"N; 30°39'44.33"E), Konyaaltı beach (36°51'41.83"N; 30°38'33.02"E), Kemer (36°35'23.07"N; 30°35'30.92"E), and Üçadalar (36°27'24.18"N; 30°33'3.43"E) of the Gulf of Antalya. During the research, jellyfish samples were taken by scuba diving with a covered bucket. A total of 44 individuals were taken in 2 samplings carried out between February and April and transported to the Akdeniz University Fisheries Faculty Research Laboratory. The weight of the transported jellyfish was measured with a scale and their mantle diameters were measured with a meter. The stock density was calculated as the number of individuals falling into the designated areas on the sea surface.

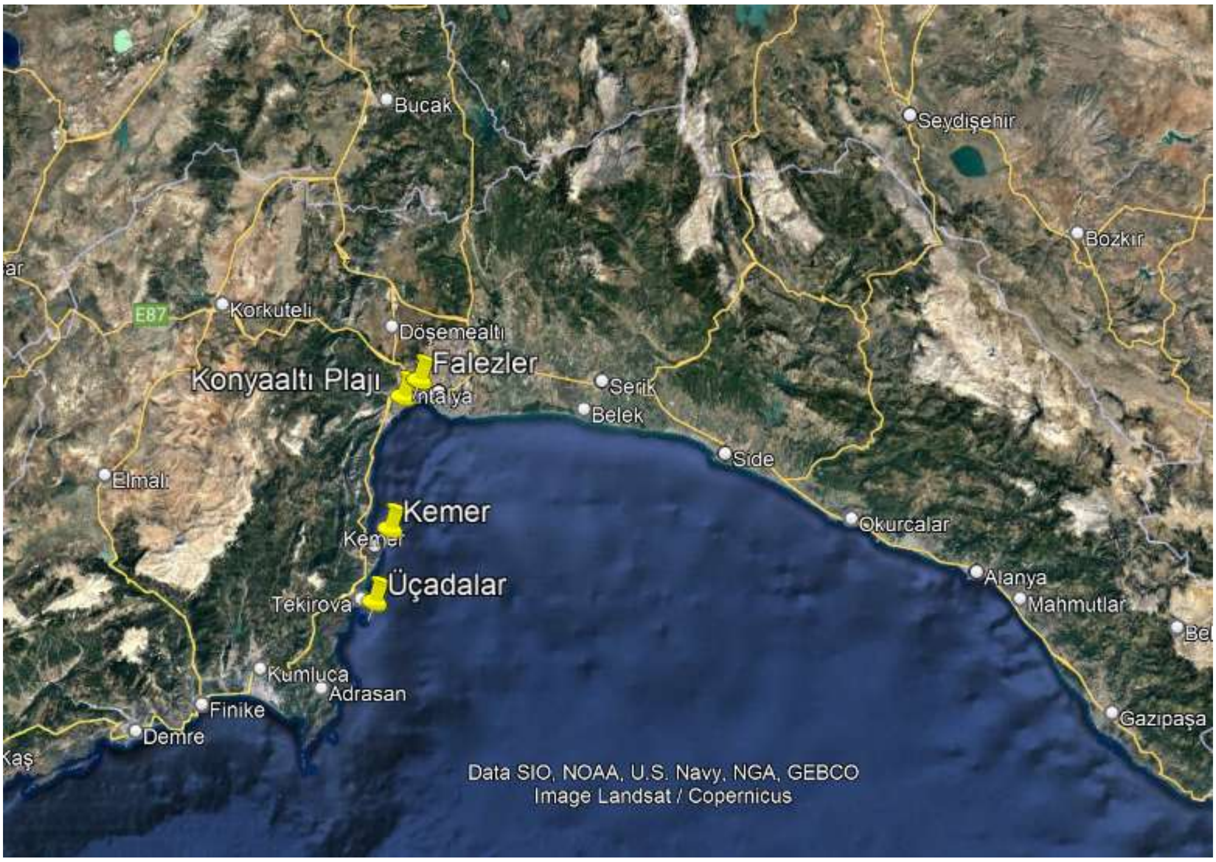


Figure 2. Stations taken jellyfish from the Gulf of Antalya

RESULTS AND DISCUSSION

Many researchers have reported that *R. nomadica*, which forms large populations in the Eastern Mediterranean, is carried westward by currents (Deidun et al. 2011; Edelist et al. 2020). In the Gulf of Antalya, this transition began in January 2024 and was completed by the end of April. The population density in the gulf reached 1 individual/10 m2 between March and April. It is thought that the reasons for this density are a warm winter season in 2023-2024 and long and strong northern winds. This density of jellyfish in Antalya, a tourist city, causes great concern. During this period when an increase in jellyfish was observed, tourism professionals constantly called our faculty and asked when the transition would be completed.

In our study, a total of 44 jellyfish were taken in 2 samplings carried out between February and April and transported to Akdeniz University, Faculty of Fisheries Research Laboratory. *R. nomadica* migrating from the Gulf of Antalya was found to have an average mass of 3780.11±304.85 g and a mantle diameter of 37.25±1.40 cm (mean±SE). Considering these values and the fact that small individuals were not detected in the gulf, it is shown that these jellyfish are individuals that reproduce in the eastern Mediterranean. Many researchers have also stated that this species reproduces in the eastern Mediterranean and is drifted westward by currents and winds (Deidun et al 2011; Zeidler et al. 2018; Edelist et al. 2020). The recent settlement and rapid spread of *R. nomadica* poses a serious threat to the ecosystem and human health (Galil, 2012; Angel et al., 2016). *R. nomadica* has become an important medusa in the eastern Mediterranean today.

CONCLUSIONS

As a result, *R. nomadica* proliferated on the coasts of Israel, Lebanon, Syria, and the Gulf of Iskenderun, poses a major problem for human health, tourism, ecology, and fishing, and is also a threat to tourism in the Gulf of Antalya and Türkiye, causing concerns. It is necessary to monitor this jellyfish, take measures to prevent its proliferation, and work to use this species for the benefit of humanity.

REFERENCES

Angel, D., Edelist, D. and Freeman, S. 2016. Local perspectives on regional challenges: jellyfish proliferation along the Israeli Mediterranean coast. Regional Environmental Change, 16: 315–323.

Avsar, D., Çevik, C., Türeli, C. 1996. İskenderun Körfezi için yeni bir tür olan (*Rhopilema nomadica*)'nın biyometrisi ve Yumurtalık Koyundaki bulunurluğu. XIII. Ulusal Biyoloji Kongresi, 17-20 Eylül 1996 İstanbul. Düzenleyen Kuruluş: İ.Ü. Fen Fakültesi, Biyoloji Bölümü.

Deidun, A., Arrigo, S., Piraino, S. 2011. The westernmost record of *Rhopilema nomadica* (Galil, 1990) in the Mediterranean – off the Maltese Islands. Aquatic Invasions 6, (Suppl. 1): S99–S103.

Edelise, D., Guy-Haim; T., Kuplik, Z., Zuckerman, N., Nemoy, P., Angel,D.L., 2020. Phenological shift in swarming patterns of *Rhopilema nomadica* in the Eastern Mediterranean Sea. Journal of Plankton Research, 42 (2): 211–219.

Galil, B.S., Spanier E., Ferguson WW. 1990. The Scyphomedusae of the Mediterranean coast of Israel, including two lessepsian migrants new to the Mediterranean. Zoologische Mededelingen, 64: 95–105

Galil, B.S. 2007 Seeing Red: Alien species along the Mediterranean coast of Israel. Aquatic Invasions, 2: 281–312,

Galil, B.S. 2012. Truth and consequences: the bioinvasion of the Mediterranean Sea. Integrative Zoology, 7: 299–311.

Kideys, A.E., Gucu, A.C. 1995. *Rhopilema nomadica*: A Lessepsian scyphomedusan new to the Mediterranean coast of Turkey. Israel Journal of Zoology, 41: 615-617.

Zeidler, W., Douek, J., Rinkevich, B., Gevili, M.G., Galil, B.S. 2018 Validation and resdescription of the hyperiidean amphipod Brachyscelus rapacoides Stephensen, 1925 (Crustacea: Amphipoda: Hyperiidea: Brachyscelidae), a new record of association with the scyphozoan jellyfish Rhopilema nomadica Galil, 1990 (Scyphozoa: Rhizostomeae: Rhizostomatidae) in the Mediterranean Sea. Zootaxa, 4471 (3): 523–534.

JUVENILES OF SCAD (*Alepes djedaba*) USING THE UMBRELLA OF THE JELLYFISH R*hopilema nomadica* AS A SHELTER

Mehmet GOKOGLU.[1*]*, Alper YILDIZ*[1]

[1] *Fisheries Faculty, Akdeniz University, Antalya Türkiye*

Corresponding author e-mail:gokoglu@akdeniz.edu.tr

ABSTRACT

Rhopilema nomadica is considered one of the most dangerous invasive species in the Mediterranean. This species negatively affects the Mediterranean ecosystem, fishing, industrial facilities, tourism, and human activities in coastal waters. It has been determined that some juvenile fish are hiding under the umbrellas of these medusas drifting westward from the coast of Antalya during the winter and spring months. Thirty-three juvenile fish were detected on the *R. nomadica* sample having a bell diameter of 33 cm and weight of 6550 g in April. In identifying the species, it was determined that these juveniles belonged to *Alepes djedab*a. The average length and weight of these juveniles were determined as 44.54 mm and 1.38 grams, respectively. The smallest juvenile was found to be 23 mm in length and 0.14 g in weight, and the largest juvenile was 74 mm in length and 4.89 g in weight. According to these results, it can be said that in the Mediterranean ecosystem, *R. nomadica* contributes to the population increase of this fish by providing shelter to the juveniles of *A. djedaba*, which is also a Red Sea migrant.

Keywords: R*hopilema nomadica, jellyfish, Alepes djadaba, shelter*

INTRODUCTION

Seven Indo-Pacific jellyfish were spotted on the Mediterranean coast of Israel in 1977, and this species was identified as *Rhopilema nomadica* (Galil et al., 1990). The species of *R. nomadica* is a native to species of the Western Indian Ocean. This jellyfish is distributed along the coasts of Pakistan, Mozambique, and the Red Sea. (Tahera and Kazmi, 2015; Zeidler et al. 2018). *R. nomadica*, which is very abundant in Israel, Lebanon, Syria, and the Gulf of Iskenderun, drifts westward from the open waters of the Gulf of Antalya between March and May with the main currents of the Mediterranean. A portion of these jellyfish also enter the Gulf of Antalya and pose concerns for human health.

The blooming event of the invasive *R. nomadica* in the eastern Mediterranean is reported to be caused by many factors such as global warming, pollution, eutrophication, overfishing, and increasing water temperature (El Regal and Temraz, 2016). This species, which forms overpopulations on the coasts of Egypt, Israel, Lebanon, and Syria, has expanded its distribution towards the western Mediterranean and reached Italy, Malta, and Tunisia (Douek et al., 2024).

It is reported that these jellyfish swarms have an oligotrophic effect on marine resources, as well as negative effects on fishing, aquaculture, industrial facilities, tourism, and human activities in coastal waters (Galil, 2007; Balistreri et al., 2017). *R. nomadica* is venomous, and the active toxic substances found in jellyfish nematocysts cause painful stings in humans, characterized by systematic symptoms that include erythematous rashes, itching, and burning sensations, as well as fever, fatigue, and myalgias (Gusman et al., 1997; Balistreri et al., 2017).

It is stated that some species of juvenile fish accompany jellyfish and shelter under their tentacles and bells (Çeviker et al., 2011; Gulşahin and Tarkan, 2011; Artuz and Tuncer, 2016).

In a study conducted by Panikar and Parasad (1952) in India, *Caranx spp*. species were observed around *Rhopilema hispidum* and was reported that juvenile fish took shelter under jellyfish bells when disturbed. Some studies, conducted in the Mediterranean, been reported that juveniles of *Alepes djedaba*, a Carangid species were seen around and under *R. nomadica*. However, no information was found about the numbers, heights, and weights of these juveniles.

This study aimed to determine the number, length, and weight of *Alepes djedaba* found under the umbrella of *R. nomadica.*

MATERIAL AND METHODS

Another research was conducted (6 April 2022) in the Gulf of Antalya (Konyaaltı Beach), where individuals of *R. nomadica* were sampled during scuba diving. The *R. nomadica* sample, taken with a lidded bucket via scuba diving from the sea at Konyaaltı beach, was transferred in seawater to the Akdeniz University Faculty of Fisheries Laboratory. During laboratory studies (measurement of weight, umbrella diameter), many juvenile fish were detected using jellyfish umbrellas as shelter. Then, the species identification of juveniles was applied, and abundance, length, and weight were measured (Figure 1, 2). In the species identification made, it was determined.

A
B

Figure 1. *R. nomadica* (A) and *A. djedaba* (B) juveniles collected from Konyaaltı beach.

RESULTS AND DISCUSSION

In this research, juvenile fish using the invasive jellyfish *R. nomadica* bell as a shelter were identified as *Alepes djeddaba*. This fish, like *R. nomadica*, is a Lessepsian species and was later included in the Mediterranean ecosystem (Golani, 1998). This fish caught with purse seines, trawls, gill nets, and fishing rods along the Turkish coast is sold in markets and consumed by consumers. In another study, we conducted on hyperiids found on *R. nomadica* in Antalya Bay, *R. nomadica* was carefully taken into a bucket by scuba diving and the lid of the bucket was closed underwater (Yılmaz and Gokoglu, 2024) (Figure 1 (A).

Some studies showed that juvenile scads (*A. djedaba*) use jellyfish umbrellas as shelter. Ohtsuka et al. (2010) identified 43 *A. djedaba* in the umbrella of *Rhopilema hispidum* with a 26 cm diameter. In previous studies on *R. nomadica,* there is no record regarding the number, length, and weight of the scad juveniles that used the jellyfish umbrella as a shelter in the Mediterranean (Gunsahin and Tarkan, 2011; Artüz and Tuncer, 2016).

In this study, the number of juveniles using the umbrella of *R. nomadica* with 33 cm diameter and 6550 g weight was found to be 33. The average length and weight of these juveniles were determined as 44.54 mm and 1.38 g, respectively. The smallest juvenile was found to be 23 mm in length and weigh 0.14 g, and the largest juvenile was 74 mm in length and 4.89 g in weight (Figure 1).

Panikar and Parasad (1952) detected 56 juvenile *Caranx calla* with lengths ranging from 8.5 mm to 18.5 mm under the umbrella of the medusa in June 1950. In this study, the smallest juvenile was found to be 23 mm and weigh 0.14 g. When this study is compared with Panikar and Parasad's study, it is seen that the juvenile fish detected are larger. *R. nomadica*, drifting westward (towards Rhodes) with currents from Iskenderun Bay, passes through the open waters of Antalya Gulf in winter and spring. During this transition, some of these jellyfish enter the gulf and cause concerns in tourists swimming in the sea. However, this transition is completed towards the summer months. During this drift, the smaller *A. djedaba* juveniles grow until they reach the Gulf of Antalya.

In conclusion, jellyfish (*R. nomadica*), which are expanded into the Mediterranean by currents, may also contribute to the distribution of this fish species (*A. djedaba*) in the Mediterranean. Since *A. djedaba*, the fish we identified in this research, will spend the critical period of its life under the jellyfish umbrella, an increase in its population can be expected.

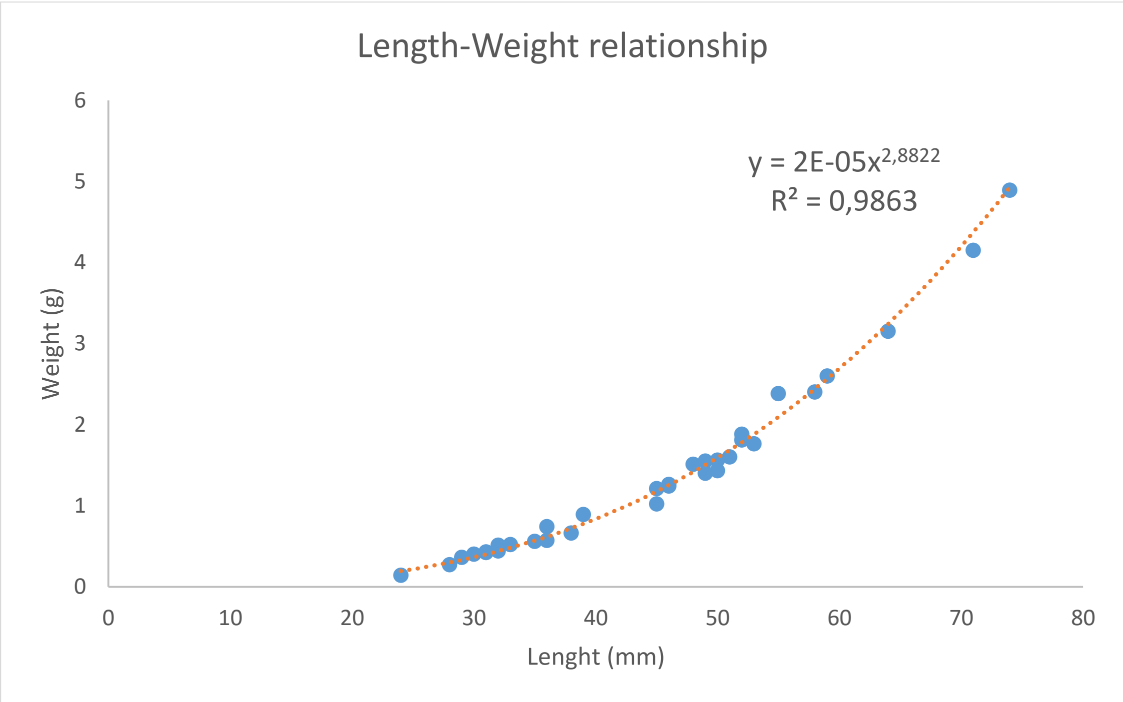


Figure 2. Length-Weight relationship of *A. djedaba* juveniles

Figure 3. Some of the *A. djedaba* juveniles using the *R. nomadica* umbrella as a shelter.

REFERENCES

Artüz, L.M., Tuncer, S. 2016. On the association of carangid juveniles, *Alepes djedaba*,with the jellyfish *Rhizostoma pulmo.* Cybium 2017, 41(1),:69-71.

Balistreri, P., Spiga, A., Deidun, A., Gueroun, S. K., Yahia, M.N.D. 2017. Further spread of the venomous jellyfish *Rhopilema nomadica* Galil, Spannier & Ferguson, 1990 (Rhizostomeae, Rhizostomatidae) in the western Mediterranean. BioInvasions Records, 6(1): 19-24.

Cevik, C., Derici, O.B., Cevik, F., Cavas, L. 2011. First record of *Phyllorhiza punctata* von Lendenfeld, 1884 (Scyphozoa: Rhizostomeae: Mastigiidae) from Turkey. Aquatic Invasions 6, Suppl. 1, S27–S28.

Douek, J., Giallongo, G., Harbuzov, Z., Galil, B.S., Rinkevich, B. 2024. Long-Term Population Genetic Features of the *Rhopilema nomadica* Jellyfish from the Israeli Mediterranean Coasts. J. Mar. Sci. Eng., 12: 171.

El Regal, M.A.A., Temraz, T.A. 2016. Blooming of the nomad jelly fish *Rhopilema nomadica* along the Egyptian Mediterranean coasts. Rapp. Comm. int. Mer Médit., 41: 490.

Galil, B.S., Spanier, E., Ferguson, W.W. 1990. The scyphomedusae of the Mediterranean coast of Israel, including two lessepsian migrants new to the Mediterranean. Zool. Meded. Leiden, 64: 95-106.

Galil, B.S. 2007. Loss or gain? Invasive aliens and biodiversity in the Mediterranean Sea. Marine Pollution Bulletin, 55: 314–322.

Golani, D. 1998. Distribution of Lessepsian migrant fish in the Mediterranean. Ital. J. Zool., 65(S1): 95-99.

Gusman, L., Avian, M., Galil, B., Patriarca, P., Rottini, G. 1997. Biologically active polypeptides in the venom of the jellyfish *Rhopilema nomadica*. Toxicology, 35: 637–648.

Günşahin, N., Tarkan, A.N. 2011. The first confirmed record of the alien jellyfish *Rhopilema nomadica* Galil, 1990 from the southern Aegean coast of Turkey. Aquatic Invasions, 6(Supplement 1): 95–97.

Tahera, Q., Kazmi, Q.B. 2015. Occurrence of *Rhopilema nomadica* Galil, 1990 (Cnidaria: Scyphozoa: Rhizostomeae: Rhizostomatidae) in Pakistani waters. Journal of Biological Research, 3 (1): 49–51.

Ohtsuka, S., Kondo, Y., Sakai, Y., Shimazu, T., Shimomura, M., Komai, T., Yanagi, K., Fujita, T., Nishikawa, J., Miyake, H., Venmathi Marani, B.A., Go, A., Nagaguchi, K., Yamaguchi, S., Dechsakulwatana, C., Srinui, K., Putchakarn, S., Mulyadi, Mujıono, N., Sutomo, Md. Yusoff, F. 2010. In-situ Observations of Symbionts on Medusae Occurring in Japan, Thailand, Indonesia and Malaysia. Bulletin of the Hiroshima University Museum, 2: 9-18.

Panıkkar N.K., Raghu Prasad R. 1952.. Interesting association of ophiuroids, fish and crab with the jellyfish *Rhopilema hispidum*. J. Bombay Nat. Hist. Soc., 51: 295-296.

Yılmaz, M., Gokoglu M. 2024. The first record of Brachyscelus rapaoides Stephan,1925 on the south of Türkiye. Mediterranean Marine Science Collective Articles A (in press).

Zeidler, W., Douek, J., Rinkevich, B., Gevili, R., Goren, M., Galil, B. S. 2018. Validation and redescription of the hyperiidean amphipod Brachyscelus rapacoides Stephensen, 1925 (Crustacea: Amphipoda: Hyperiidea: Brachyscelidae), a new record of association with the scyphozoan jellyfish Rhopilema nomadica Galil, 1990 (Scyphozoa: Rhizostomeae: Rhizostomatidae) in the Mediterranean Sea. Zootaxa 4471(3), 523-534.

SEAFOOD FRAUD: ITS ASPECTS ON TRADE, MARKETING AND FOOD SAFETY

Nalan GÖKOĞLU[1]

[1]*Akdeniz University Fisheries Faculty, Antalya, Türkiye*
Corresponding author e-mail: ngokoglu@akdeniz.edu.tr

ABSTRACT

Intentional manipulation of the structure of foods for economic gain is called food fraud. In addition to causing economic losses for food businesses, food fraud also causes losses to the image of countries in exporting products and poses a risk to consumer safety. Because the species have similar appearances, seafood products are susceptible to counterfeiting. Mislabeling, species substitution, species adulteration, overglazing of frozen products, short weighting, and undeclared additives are the main food fraud examples in seafood. There is a need to combat food fraud effectively. For this, national food programs should be strengthened, new regulations should be developed, and intergovernmental cooperation should be increased.

Keywords: Fraud, Seafood, Food safety, Economic loss

INTRODUCTION

Food fraud is generally defined as illegal deception for economic gain using food (Spink and Moyer, 2011; Spink et al., 2014; Spink et al., 2016; Wu et al., 2017, Spink et al., 2019). Food fraud is a phenomenon that can occur at all stages of the supply chain and on an international scale. Concerns, awareness, and dangers about food fraud are increasing. According to FAO (2021), food fraud is any suspected intentional action committed when a food business operator intentionally decides to deceive customers about the quality and/or content of the food they are purchasing to gain an economic advantage. Within the definitions for food fraud, the motivation or driving factor for economic gain is generally agreed (Robson et al., 2021). Food fraud has been conducted since antiquity. It was similar to those occurring in modern times, the scale then was limited and covered a small geographic area. Because modern food supply chains have been lengthened, complicated, and accelerated, the risk of food fraud has broadened to include entire global populations (Spink and Moyer, 2011). Food fraud is an issue affecting all food supply chains and therefore the entire food industry, customers, and consumers. Food supply chains are increasingly vulnerable due to globalization and lengthening of supply chains (Lotta & Bogue, 2015; Trivedi et al., 2016). Food fraud is committed in different ways. These include unauthorized production, mislabeling, misbranding, and intellectual property rights fraud. Adding sugar to honey is an example of this. Food fraud is multifaceted and complex. FAO (2021) has reported that food fraud cases reduce consumers' trust and cause them to view even safe products with suspicion. The same report also states that the success of food fraud may increase the likelihood that fraudsters will take greater risks on food, which in turn may pose a threat to the security and integrity of food supply chains. There are records of fraud for many foods, including cereals, dairy products, fruits and juices, meat and seafood, nuts, spices, organic foods, and honey (Manning, 2016). Despite technological advances that enable the detection of food fraud and consumers' sensitivity to food safety, it is stated that approximately 10% of the food on supermarket shelves in the USA is subject to fraud. Nowadays, food fraud is also quite common in Europe (Meerza et al., 2019).

FOOD FRAUD AND FOOD SAFETY

Food fraud could pose a food safety risk. With globalization, the fact that the food supply is long, complex, and often difficult to trace, insufficient traceability increases food fraud and, as a result, creates potential safety and health risks. Food safety risks in food fraud are generally unintentional and not intended to harm human health. It includes several common chemicals or pathogens that are generally known to pose public health hazards (WHO, 2016; Spink et al., 2017). Food adulteration poses a significant public health risk associated with food safety issues in the food market. Most food items on the market contain varying degrees of additives. These substances include chemicals, products that are not original ingredients of foods, low-quality products, and physical or inert substances (Gizaw, 2019).

ECONOMIC IMPACTS OF FOOD FRAUD

Food fraud can also result in severe economic consequences and can damage national reputations in the global food market. Food fraud is estimated to cost the global food industry $40 billion annually and $10 to $15 billion per person. The economic loss of a food scam may include costs of product recall or withdrawal, incident investigation, liabilities, lost sales, and share price decline. Current costs for companies include conducting a food fraud vulnerability assessment plan. A single food fraud scandal can cause long-term industry-wide losses, destroy valuable brands, close export markets, and damage trust in public institutions. Significant investment is required to obtain effective strategies for supply chain risk. (Galvin-King et al., 2018).

TRADE AND MARKETING ASPECTS OF FOOD FRAUD

The presence of many buyers and sellers in supply chains also increases the opportunities for fraud in the markets. Fraud can occur, especially when supplied from countries with weak or inadequately enforced local regulations or poorly structured legal systems. Failure to develop food fraud prevention strategies, wide supply chains, and difficulties in detecting fraud increase the risk of fraud in international trade. Food fraud can increase costs in trade. Because as food fraud increases, it increases the need for border inspections and testing and the costs of food safety. This increases the costs for both the buyer and the seller. Detecting and preventing food fraud is costly. The measures manufacturers take to demonstrate product authenticity, increase supply chain security, and demonstrate compliance with product and process standards are costly (Ehmke et al., 2019).

SEAFOOD FRAUD

Seafood fraud encompasses any illegal activity that misrepresents the fish you purchase, including mislabeling or substituting one species for another. Seafood is highly susceptible to fraud due to factors such as the similar appearance of many species, increasing global trade, and varying quality, supply, and demand (Silva et al., 2021). However, without distinct morphological indicators, it can be hard to visually determine whether a seafood product has been fraudulently labeled. Among food commodities, seafood has been identified as a commodity particularly vulnerable to misrepresentation. For example, the European Parliament identified fish and fish products as the second highest category of foods at risk for fraud (Kroetz et al., 2020). Each type of seafood fraud confers economic gain on the fraudster, either by selling a lower-quality product at a higher price or by avoiding costs. The extent of fraud in the seafood sector is difficult to ascertain because much fraudulent activity likely goes undetected. However, several studies in multiple countries have uncovered high rates of mislabeled seafood species. Given the significant seafood market share associated with popular seafood products, even low rates of some mislabeled products may result in consumers purchasing significant quantities of fraudulent seafood.

TYPES OF FRAUD IN SEAFOOD

The most common type of fish fraud involves intentional mislabeling and species substitution. To a lesser extent, fraud occurs when fish is overglazed or overbreaded, leading to deceiving consumers regarding the nature of fishery products. Undeclared use of water-binding agents is also a fraudulent practice that leads to increasing the weight of products and selling additional water substituted for fish.

Mislabeling

Mislabeling and species substitution have an important place among the most common frauds in seafood. This means that the consumer buys a different species instead of the one listed on the label. (Acutis et al., 2019). Seafood mislabeling has dire consequences, including compromising sustainable fisheries and undermining conservation efforts. Mislabeling can also create health risks for consumers. In recent years, the increase in seafood mislabeling fraud has increased concerns about the identity and safety of these products. (Kroetz et al., 2020).

Species substitution

Species substitution occurs when low-value or less-desirable fish species are swapped for more expensive varieties, for example, the fraudulent marketing of farmed salmon as wild-capture species. For instance, the substitution of whiting for haddock, coley for cod, dab for plaice or sole, and bigeye tuna for yellowfin tuna are just a few examples. The flesh of many fish species is similar in appearance, taste, and texture. It can be difficult to identify or differentiate species once these have been processed or prepared for consumption and presented with flavoring in sauces or batter. Species substitution can also occur where a higher-value species is marketed as a lower-value species to avoid taxation. It can also occur to conceal the geographical origin or to hide an illegally harvested protected species or a species from a protected area.

Species adulteration

Species adulteration is different from species substitution. In species adulteration, undeclared species are partially added to a named product. For example, a lower-value species is added to the declared species to add weight.

Overglazing of frozen products

Glazing seafood is a common practice used in the industry that not only aids in reducing product weight loss by dehydration but also prevents freezer burn by surrounding the product in a thin layer of ice. Although there is no exact standard, 5-10% is considered sufficient for most frozen seafood products. By over-glazing, the weight of the products is artificially increased, and consumers receive less product than expected while paying for extra ice.

In a study, it was determined that 7 of 111 frozen fish fillets collected from markets were over-glazed (>10% glazed) and 10 were short-weight (Peterson et al., 2021). In another study, Country of Origin Labeling (COOL) compliance, species authentication, acceptable market names, net weights, and percent glaze of shrimps were examined and as a result, 26% of the samples contained more than 20% glaze and 37% were found to have short weights 0and 37% were found to have species labeling errors (Rivers et al., 2024).

Short weighting

Less known, but far more common, is short-weighting. This is when processors misrepresent the weight of a seafood product through practices such as overglazing, soaking, and breading.

Processors will often add a layer of ice or a preservative to keep a seafood product fresh, a normal and legal practice. However, when a processor uses excess ice (overglazing) or additives (soaking) and includes that weight with the net weight of the seafood, that's fraud. Consumers should pay for the weight of the seafood alone. Short-weighting charges consumers more for less seafood.

Undeclared food additives

Sometimes salt or other chemical additives may be used to reduce the fish content of the product. Additives are added to the products by dipping and injection methods. This kind of methods are used to increase the water retention capacity of fish muscle and produce products with low fish content. Gel injection is performed to increase the weight of the shrimp and provide an attractive appearance. Since the injected products may be harmful to health, they may pose a risk to human health. For example, phosphates used to improve some properties of seafood can characterize economic fraud when used incorrectly or excessively (Gonçalves and Ribeiro, 2008).

CHALLENGES IN DETECTING AND PREVENTING FOOD FRAUD

1- One of the challenges is detecting the unexpected.
2- The issue of food fraud and its place in marketing is not fully understood and there is a need for a legal definition on this issue.
3- It may be difficult or impossible for consumers to detect food fraud at supermarkets or market stalls. Unless the product poses an immediate health hazard, consumers may not necessarily know, even after consuming the product that they were victims of food fraud.
4- All substances used to add food cannot be easily detected through food safety and quality tests.
5- Species identification of fish and seafood products is difficult because the morphological features that can usually be identified have been removed during processing steps such as cooking, smoking, salting, and canning.
6- Species substitution and mislabeling are difficult to detect when fish morphological features such as heads, tails, and fins are removed and fish are processed into fillets, ready-to-eat breaded or battered products, or highly processed in pre-prepared fish meals (FAO, 2018).

CONCLUSIONS

Fish traceability is key to combating fish fraud, enforcing food safety regulations, and ensuring high standards of sustainable fisheries management. Traceability is also critical for ensuring the quality of fish products and minimizing health risks for consumers. Close collaboration between all these different government agencies is essential for an effective response. There is a need to strengthen official national food control programs by developing new regulations to combat fish fraud; enhancing enforcement activities prohibiting landings and market access for products from illegal, unreported, and unregulated fishing; introducing monitoring and surveillance programs for assessing the degree of compliance with fish labeling regulations; and upgrading laboratory detection methods based on DNA barcoding (FAO, 2018).

REFERENCES

Acutis, P.L., Cambiotti, V., Riina, M.V., Meistro, S., Maurella, C., Massaro, M., Stacchini, P., Gili, S., Malandra, R., Pezzolato, M., Caramelli, M., Bozzett, E. 2019. Detection of fish species substitution frauds in Italy: A targeted National Monitoring Plan. Food Control, 101:151-155.

Ehmke, M.D., Bonanno, A., Boys, K., Smith, T.G. 2019. Food fraud:economic insights into the dark side of incenize. Australian Journal of Agricultural and Resource Economics, 63: 685–700.

FAO. 2021. Food fraud – Intention, detection and management. Food safety technical toolkit for Asia and the Pacific No. 5. Bangkok.

Gizaw, Z. 2019. Public health risks related to food safety: 68.

Hong, E., Lee, S.Y., Jeong, J.Y., Park, J.M., Kim, B.H., Kwon, K., Chun, H.S. 2017. Modern analytical methods for the detection offood fraud and adulteration by food category. Journal of the Science of Food and Agriculture, 97: 3877–3896.

Kroetz, K., Luque, G.M., Gephart, J.A., Jardine, S.L., Lee, P., Moore, K.C., Cole, C., Steinkruger, A., Donlan, C.J. 2020. Consequences of seafood mislabeling for marine populations and fisheries management. PNAS, 117 (48:0318–30323.

Lotta, F., Bogue, J. 2015. Defining food fraud in the modern supply chain. European Food and Feed Law Review, 10: 114.

Manning, L. 2016. Food fraud: policy and food chain. Opinion in Food Science, 10:16-21.

Meerza, S.I.A., Giannakas, K., Yiannaka, A. 2019. Market and welfare effects of food fraud. *Australian Journal of Agricultural and Resource Economics,* 63: 759-789.

Robson, K., Dean, M., Haughey, S., Elliott, C. 2021. A comprehensive review of food fraud terminologies and food fraud mitigation guides. Food Control, 120: 107516.

Spink, J., Moyer, D.C. 2011. Defining the public health threat of food fraud. Journal of Food Science, 76(9): 157– 163.

Spink, J., Moyer, D.C., Park, H., Heinonen, J. 2014. Development of a counterfeit incident clustering tool. Crime Science, 3: 1-8.

Spink, J., Moyer, D.C., Speier-Pero, C. 2016. Introducing the Food Fraud Initial Screening model (FFIS). Food Control, 69: 306-314.

Spink, J., Hegarty, P.V., Fortin, N.D., Elliott, C.T., Moyer, D.C. 2019. The application of public policy theory to the emerging food fraud Risk: Next steps. Trends in Food Science & Technology, 85:116–128.

Trivedi, D.K., Hollywood, K.A., Rattray, N.J., Ward, H., Trivedi, D.K., Greenwood, J., et al. 2016. Meat, the metabolites: An integrated metabolite profiling and lipidomics approach for the detection of the adulteration of beef with pork. Analyst, 141(7): 2155–2164.

WHO, World Health Organization. 2016. Melamine Accessed September 2016.

Wu, Y., Miao, H., Shao, B., Zhang, J., Spink, J., Moyer, D. 2017. Food Fraud, In: Food Safety in China – Past, Present, and Future, Science, Technology, Management and Regulation, Editors Jwu-shan J.J., Chen J. (eds). Wiley & Sons, p. 253-270

PREVENTION OF MELANOSIS IN SHRIMP WITH THE COMBINATION OF ANTIMELANOTIC AGENTS AND VACUUM PACKAGING

Nalan GÖKOĞLU[1]

[1]*Akdeniz University Fisheries Faculty, Antalya, Türkiye*
Corresponding author e-mail: ngokoglu@akdeniz.edu.tr

ABSTRACT

Rich protein and low-fat content make shrimp a valuable food. Shrimps have a short shelf life due to microbial spoilage and melanosis. Melanosis is an enzymatic phenomenon in shrimps that reduces their quality and market value. For melanosis, which develops quickly, the necessary prevention method should be applied immediately after catching it. Various techniques and mechanisms have been developed to prevent enzyme activity. The most common and most effective method is the use of sulfites. However, the fact that sulfite causes allergic reactions has led researchers to develop new methods. This study investigated the effects of using grape seed extract and sodium metabisulfite combined with vacuum packaging on melanosis formation in shrimp (Aristaeomorpha foliacea). The shrimps, which were obtained from the fishermen immediately after being caught, were treated with grape seed extract and sodium metabisulfite, and packaged with and without vacuum. Packaged shrimps were stored at 4°C. Melanosis development and color values (L*, a*, b*) were measured during storage at daily intervals. Grape seed and sodium metabisulfite applications were effective in delaying melanosis. Additionally, vacuum packaging showed a synergistic effect with antimelanotic agents in preventing melanosis. Compared to the control group, the most effective application was the sulfite vacuum combination, followed by the grape seed vacuum combination.

Keywords: Shrimp, Melanosis, Prevention, Grape seed, Antimelanotic

INTRODUCTION

Shrimps have an important place in human nutrition. In addition to being rich in protein, shrimp meat is low in calories due to its low fat content. Shrimps, which are caught all over the world, have a significant export potential. A significant portion of the caught shrimp is offered to the market fresh and unprocessed. However, shrimp are perishable, and their shelf life is extremely limited. Its structure suitable for microbial development accelerates the deterioration process. Shrimps have a short shelf life and are extremely perishable due to microbial spoilage and melanosis (Gokoglu 2004; Martinez-Alvarez *et al.* 2005 a; Benner *et al.* 1994). Darkening called "melanosis" or "Black Spot", which begins immediately after catching and occurs enzymatically, is one of the factors limiting shelf life. During melanosis, phenols are oxidized to quinones by the polyphenol oxidase enzyme. This mechanism is followed by non-enzymatic polymerization of quinones, and this event causes the formation of high molecular weight and dark or black pigments (Montero *et al*. 2001; Arias *et al*. 2007). Blackening occurs shortly after fishing and is a very important problem in the shrimp industry. Melanosis develops very quickly in shrimps stored in the cold without being treated with any preservatives and can significantly change their market value within 24 hours (Rotllant *et al*., 2002). Although it is not harmful to consumer health, it negatively affects the acceptability and reduces the market value of the product (Montero *et al*., 2001).

Developing methods to preserve the quality of this valuable food product for a longer period or improving existing methods are among the main expectations of the relevant sector.

Various techniques and mechanisms have been developed for many years for this undesirable enzyme activity. These techniques attempt to eliminate one or more of the essential components (oxygen, enzyme, copper, or substrate) from the reaction (Gokoglu and Yerlikaya 2008). Physical methods and preservatives are used for enzymatic and non-enzymatic browning (Hardisson et al. 2002). Sulfites are the most effective and multi-purpose agent in controlling enzymatic browning in shrimp. The use of sulfites is gradually decreasing because it is known that sulfites cause some health problems such as asthma and allergic reactions (Gokoglu and Yerlikaya 2008; Villamiel 2006). Grape seed has strong antioxidative activity. Its antioxidative activity is related to its ability of radical scavenging, metal chelating, and synergism with other antioxidants.

In this study, it was aimed to investigate the effects of natural antioxidant source grape seed extract and vacuum packaging on preventing shrimp browning. To determine the effect of grape seed extract, it was compared with sodium metabisulfite, which is widely used to prevent melanosis.

MATERIAL AND METHODS

Giant red shrimp (*Aristaeomorpha foliacea*) was used as material in the study. Freshly caught shrimps were transported to the laboratory with ice in Styrofoam boxes without any protective chemical application. Commercial grape seed extract (5%) and sodium metabisulfite (1.25%) solutions were prepared with distilled water. In the laboratory, shrimps were divided into three groups (GS = Grape seed extract treated group; S = Sodium metabisulfite treated group; C = Without treatment group (Control). Shrimps were dipped in grape seed extract and sodium metabisulfite solutions for 1 minute. These groups were placed in polyethylene bags and then packed with and without vacuum. The packages were stored in the refrigerator at 4°C. During storage, color measurements were made in the samples and the development of melanosis was examined.

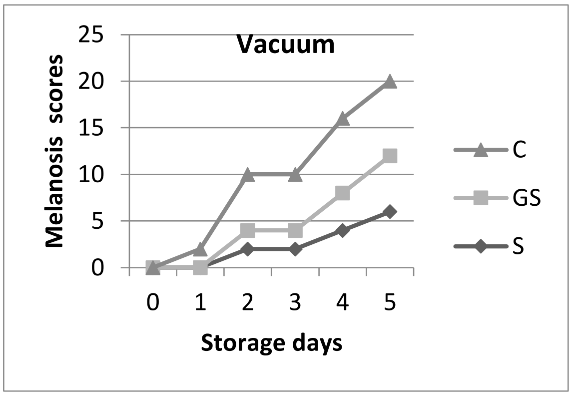
A chromometer instrument (CR 200 Konica Minolta, Osaka, Japan) was used for color measurement. Color was measured in 3 different areas on the shrimp shells and the results were given as average values. L*(brightness), a*(redness), and b*(yellowness) values were measured in the samples. Melanosis was assessed as sensory, and the scale developed by Otwell and Marshall (1986) was used for this purpose (Table 1).

Table 1. Scale for melanosis evaluation (Otwell & Marshall 1986).

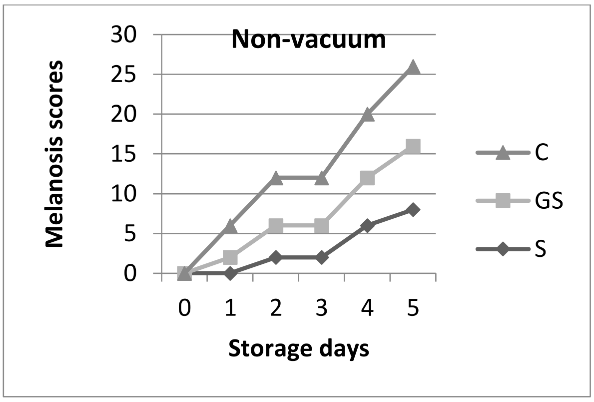
Melanosis scores	Description
0	Absent
2	Slight, noticeable on some shrimp
4	Slight, noticeable on most shrimp
6	Moderate, noticeable on most shrimp
8	Heavy, noticeable on most shrimp
10	Heavy, totally unacceptable

RESULTS AND DISCUSSION

It was determined that the application groups had significant ($p<0.01$) effects on melanosis scores. The highest scores were detected in untreated control samples. The lowest scores were determined in shrimp samples treated with sodium metabisulfite (Figure 1). Storage time had significant ($p<0.01$) effects on melanosis. Melanosis scores, which were initially "0", were determined as 6, 6, and 8 in vacuum-packaged S, GS, and C samples, respectively, at the end of the 5-day storage period, while they were determined as 8, 8, and 10 in non-vacuum samples. While the highest melanosis scores were determined in the control group at the end of the storage period, no difference was observed between the scores of sulfites and grape seed-treated samples. Sulfite is considered the best practice in preventing browning in shrimp. Metabisulfite has been reported to reduce quinones to phenols, react with quinones to give colorless compounds, or act as an irreversible PPO inhibitor, and therefore more effective in preventing melanosis (Lambrecth 1995; Ricquebourg *et al.* 1996). Bisulfites show competitive inhibition by binding sulfhydryl groups of the active part of the enzyme polyphenol oxidase. Bisulfide inhibition depends on the reaction of the sulfides with the quinones and results in the formation of irreversibly inhibited sulfoquinone forms of polyphenol oxidase (Kim *et al*. 2000). Grape seed extracts are rich in polyphenolic compounds. They are also used as food additives due to their antioxidant activity. It has also been reported by other researchers that grape seed extract inhibits melanosis (Gokoglu and Yerlikaya 2008; Sun et al. 2014).



(a)



(b)

Figure 1. Melanosis scores of vacuum (a) and non-vacuum (b) packaged application groups

In our study, it has been shown that grape seed extract can be used as an alternative to sulfite. Additionally, the extra effect of vacuum packaging in preventing melanosis has also been determined. When the color measurement analysis results were evaluated, the highest ($p<0.05$) brightness (L*) values were obtained with sulfite application. No difference was observed in the L* value between grape seed extract and control samples. While L* values increased in the first 3 days of storage, they showed a significant ($p<0.05$) decrease in the following days (Table 2). Vacuum packaging had no significant effect on L* values. Grape seed extract and sulfite applications significantly affected the a* value (redness). The highest a* value was determined in samples treated with grape seed extract. There was no significant difference between the sulfite-treated group and the control group. A significant ($p<0.01$) increase in a* values was detected with storage. Vacuum packaging also had a significant ($p<0.01$) effect on the a* value. Higher * values were obtained in vacuum-packaged samples. Effects of sulfite and grape seed extract applications on the b* values of shrimps were not significant. While a significant decrease was observed in b* values at the beginning of storage, a decrease was determined in the following days of storage. A significant ($p<0.01$) effect of packaging on b* values was observed (Table 2). Higher b* values were found in vacuum-packaged samples than in non-vacuum samples.

Table 2. Duncan Multiple Comparison Test results for color (L*, a*, b*) values[1,2,3]

Factors	L*	a*	b*
Applications			
S	36.3642a	12.5920 b	7.0279 a
GS	35.7468 ba	14.6634a	7.0890 a
C	34.4688 b	12.0092 b	6.6418 a
Storage days			
0	34.101 c	13.3080 b	7.3840 b
1	37.757 a	11.3070 c	5.3757 d
2	38.446 a	12.9717 b	6.3457 c
3	36.318 ba	13.5857 b	6.6790 cb
4	34.684 bc	11.6260 c	7.3257 b
5	31.922 d	15.6617 a	8.4053 a
Packaging			
Vacuum	35.1273 a	13.7906 a	7.2159 a
Non-vacuum	35.9122 a	12.3628 b	6.6226 b

[1] Each number represents the mean ± standard deviation.
[2] S= Sodium metabisulfite.; GS= Grape seed extract.; C= Control
[3]Means within the same factor and the same column with different letters (a, b, c, d) are different at $p < 0.01$ and $p < 0.05$ levels.

CONCLUSIONS

Sodium metabisulfite and grape seed extract applications were effective in preventing the darkening phenomenon called melanosis in shrimps. Since sulfite applications cause allergic reactions in humans, it has been determined that grape seed extract, a natural herbal extract, can be used as an alternative. In addition to these applications, vacuum packaging also showed a synergistic effect with antimelanotic agents in preventing melanosis. Any application that will prevent or delay the darkening phenomenon called melanosis in shrimp, which is a product of high economic value, will prevent both product losses and economic losses in the shrimp industry.

REFERENCES

Benner, R.A., Miget, R., Finne, G., Acuff, G.R. 1994. Lactic acid/melanosis inhibitors to improve the shelf life of brown shrimp *(Penaeus aztecus*). Journal of Food Science, 59 (2): 242-245.

Gokoglu, N. 2004. The effect of organic acid treatments on melanosis inhibition and shelf life in shrimp (Penaeus japonicus). Acta Alimentaria, 33(2): 203-211.

Gokoglu, N., Yerlikaya, P. 2008. Inhibition effects of grape seed extracts on melanosis formation in shrimp (Parapenaeus longirostris). International Journal of Food Science and Technology, 43: 1004-1008.

Hardisson, A., Rubio, C., Frías, I., Rodríguez, I., Reguera, J.I. 2002. Content of sulfite in frozen prawns and shrimps. Food Control, 13: 275-279.

Kim, J., Marshall, M.R, Wei, C. 2000. Polyphenoloxidase. In: "Seafood enzymes: Utilization and influence on postharvest seafood quality", Marcel Dekker New York, p. 271-315.

Lambrecht, H.S. 1995. Sulfite substitutes for the prevention of enzymatic browning in foods. In: "Enzymatic Browning and Its Prevention", Lee C.Y, and Whitaker J.R. (eds), p. 313-323.

Martinez-Alvarez, O., Lopez-Caballero, M.E., Montero, P. Gomez-Guillen, M.C. 2005. A 4-hexylresorcinol-based formulation to prevent melanosis and microbial growth in chilled tiger prawns (*Marsupenaeus japonicus*) from aquaculture. Journal of Food Science, 70: M415–M422.

Montero, P., Avalos, A., Perez-Mateos, M. 2001. Characterization of polyphenol oxidase of prawns (*Penaeus japonicus*). Alternatives to inhibition: additives and high-pressure treatment. Food Chemistry, 75: 317- 324.

Otwell, W.S., Marshall, M.R. 1986. Screening alternatives to sulfating agents to control shrimp melanosis (black spot), Florida Sea Grant Technical Paper, 46, 1-20.

Ricquebourg,,S.L., Robert-Da Silva, C.M.F., Rouch, C.C., Cadet, F.R. 1996. Theoretical support for a conformational change of polyphenol oxidase induced by metabisulfite. Journal of Agricultural and Food Chemistry, 44: 3457–460.

Rotllant, G., Arnau, F., Garcia, J.A., Garcia, N., Rodriguez, M., Sarda, F. 2002. Note. Effect of metabisulphite treatments and freezing on melanosis inhibit in rose shrimp *Aristeus antennatus* (Risso, 1816). Food Science Technology International, 8(4): 243-247.

Sun, H., Lv, H., Yuan, G., Fang, X. 2014. Effect of grape seed extracts on the melanosis and quality of Pacific white shrimp (Litopenaeus vannamei) during Iced Storage. Food Science and Technology Research, 20 (3): 671-677.

Villamiel, M., Del Casillo, M.D., Corzo, N. 2006. Browning Reactions. In: "Food Biochemistry and Food Processing", Hui Y.H. (Ed.) Blackwell Publishing, p. 56-83.

APPLICATION OF WATER TREATMENT METHODS IN REMOVAL OF ENVIRONMENTAL RESIDUAL ANTIBIOTICS

Guzin CERAN[1], Nazli TURKTEN[2], Yunus KARATAS[2]

[1] Kirsehir Ahi Evran University, Faculty of Medicine, Department of Anaesthesiology and Reanimation, 40100, Kirsehir, Turkiye
[2)] Kirsehir Ahi Evran University, Faculty of Arts and Sciences, Department of Chemistry, 40100, Kirsehir, Turkiye.
Corresponding author e-mail: nazli.turkten@yahoo.com

ABSTRACT

Antibiotics are chemical substances that have specific actions for microorganisms and have been commonly used for many years in different fields such as medicine, veterinary medicine, aquaculture, and agriculture. The presence of residual antibiotics in the environment can act as a microbial inhibitor, leading to adverse effects on the life activities of animals and plants in the ecosystem. As a result of the limited absorption capability of antibiotics through animal intestines, the excessive release of veterinary antibiotics to the environment has become an urgent concern to meet, particularly in countries with extensive animal husbandry. Furthermore, environmental residual antibiotics can pose a potential direct or indirect threat to human health resulting in various symptoms (allergic shock, neurological toxicity, cardiotoxicity, mitochondrial toxicity, etc.). Conventional methods including physical, chemical, and biological treatments are not capable of large-scale removal of antibiotics from water, and these methods can cause secondary pollution. Therefore, efficient strategies are needed relying on the elimination of persistent and stable antibiotic residues from water. Advanced oxidation processes (AOPs) are environmentally friendly technologies currently under development and based on the generation of hydroxyl radicals as powerful oxidizing agents. This study summarized a comprehensive overview of the implemented water treatment methods, primarily AOPs in removing antibiotics, and discussed potential treatment solutions for future research.

Keywords: Advanced oxidation processes, antibiotics, Fenton, ozonation, photocatalysis.

INTRODUCTION

Antibiotics are medicines especially active against bacterial infections with specific actions on microorganisms that can decrease or further stop the multiplication of these microorganisms. The clinical usage of antibiotics is not only limited to treating infectious diseases but also extends to modern medical treatment procedures such as organ transplants and open-heart surgeries. Unfortunately, the misuse of this modern medicine has caused a rapid increase in antimicrobial resistance and rendered some infections effectively untreatable with clinically approved almost all known antibiotics (Hutchings et al., 2019; Khmaissa et al., 2024).

Antibiotics are probably the most effective class of drugs in human medicine, and they are also widely used in animal husbandry as veterinary antibiotics including penicillin, macrolides, tetracyclines, and sulfonamides. The widespread use of antibiotics in animal husbandry can be detected in animal manure, and they can lead to the growth of antibiotic-resistant bacteria (ARB) in agricultural soil and end up in surface and groundwater. Additionally, antibiotic residues in the environment act as microbial inhibitors causing considerable adverse outcomes on animal life activities and plants. These structural and functional impacts on microbial communities can result in direct toxic effects on the plants and follow alterations in the carbon and nitrogen cycle that microorganisms participated in. Besides, the extensive antibiotic accumulation in the environment causes a remarkable increase in ARB and antibiotic-resistance genes (ARG), posing a potential threat to global human health. Among drug-resistant infections, antibiotic-resistant bacteria infection can cause notable damage to the control system, and even large intakes of antibiotics can induce intestinal diseases associated with the imbalance of the microbial community in the gastrointestinal tract (Conco et al., 2022; Khmaissa et al., 2024; Yang et al., 2024). An estimation was reported that 10 million people will die annually by 2050 unless action is taken due to alarming severe drug resistance (O'Neill, 2016).

Recently, scientific research has begun to address the remediation of antibiotics in wastewater. In this regard, modern wastewater treatment plants are essential barriers preventing the release of antibiotics and associated effluents (ARB and ARG) identified as contaminants of emerging concern (CECs) in water systems (Drane et al., 2024; Godoy and Sánchez, 2020). The study aimed to assess the water pollution caused by traces of antibiotics and also suggested innovative water treatment solutions.

WASTEWATER TREATMENT PLANTS

Wastewater treatment plants (WWTPs) use a variety of technologies, classified as primary, secondary, and tertiary treatment methods, to treat wastewater containing antibiotic traces.

Primary wastewater treatment is a physical screening and settling step to remove large solid particles and involves units of screens and primary clarifiers. The process is called chemically enhanced primary treatment since coagulants (aluminum salt, ferric iron salt, polymers, etc.) are added in primary units. Moreover, additional chemicals should be considered to prevent salting problems. Adsorption is a common primary treatment process applied to eliminate non-traditional pollutants from WWTPs and transfer them to another medium with incomplete degradation (Drane et al., 2024; Garrido-Cardenas et al., 2020; Zhang and Li, 2011). Granular activated carbon and powdered carbon are not only used in the removal of antibiotics in WWTPs but also commonly applied in drinking water treatment plants (Berges et al., 2021).

The secondary step is predominantly characterized by biological treatment methods such as aerobic and/or anaerobic processes. Activated sludge-loaded WWTPs highlighted that sludge as an adsorbent presents a challenge in separating sludge from an aqueous phase depending on varying seasons. Furthermore, the operational parameters of WWTPs containing sludge loading rate and hydraulic retention time affect the removal efficiency of toxic chemical pollutants. This implies that secondary wastewater treatment is specific to WWPTs, and each case study should be assessed individually (Drane et al., 2024; Wang et al., 2024).

The disinfection processes such as chlorination, ultraviolet (UV) radiation, ozonation, and membrane filtration constitute tertiary wastewater treatment (Drane et al., 2024). Zhuang et al. (Zhuang et al., 2015) have studied three disinfection methods (chlorination, UV radiation, and ozonation) for the inactivation of ARG (sul1, tetG, and intI1) on a laboratory scale and reported that chlorination and UV radiation process achieved a better performance than ozonation.

The effectiveness of WWTPs especially depends on the specificity of CECs and the usage pattern of CECs (Moles et al., 2024). The concentration, type, and detection frequency factor of CECs detected in WWTPs differ significantly in terms of consumption levels and usage patterns (Wang et al., 2022). Antibiotic removal efficiencies of a series of antibiotics in sewage treatment plants have been investigated and reported that removal efficiency is related to antibiotic concentrations in the effluent (Li et al., 2024). Another study on the removal efficiency of CECs among various WWTPs highlighted the inconsistency of the complex selection pressure between antibiotic and ARG and also the crucial role of mobile genetic elements in the spread of antibiotic resistance (Zhang et al., 2024b).

ADVANCED OXIDATION PROCESSES

Advanced oxidation processes (AOPs) are highly efficient and competitive oxidizing technologies for water and wastewater treatment. These novel methods are considered a promising solution to the mineralization of CECs and are mainly used in bench and pilot scales. The mechanism of AOPs is based on the generation of reactive oxygen species such as hydroxyl radicals, superoxide radicals, and hydroperoxyl radicals.

The most extensively used AOPs for eliminating antibiotics from pharmaceutical wastewater are related to photolysis under UV irradiation, homogeneous photocatalysis with Fenton reagent, heterogeneous photocatalysis, and ozonation. Furthermore, these processes can be applied by combining different varieties of AOPs (H_2O_2/O_3/UV) and combinations with other treatment methods (adsorption/O_3, activated carbon adsorption//O_3/membrane filtration) (Garrido-Cardenas et al., 2020; Godoy and Sánchez, 2020; Michael et al., 2013; Moles et al., 2024).

Ozonation and granular activated carbon adsorption techniques are combined to remove ARG and organic pollutants (Yang et al., 2019). Moreover, O_3/peroxymonosulfate (PMS) (Deniere et al., 2022; Lu et al., 2022) and O_3/PMS/biological activated carbon (Zhang et al., 2024a) processes are hot topics on the removal efficiency of antibiotics and limited research has been reported until now. A tertiary wastewater treatment study (H_2O_2/UV-C, PMS/UV-C, and PMS/Fe(II)/UV-C) is applied for the removal of antibiotics and ARG from a full-scale WWTP in Spain (Rodríguez-Chueca et al., 2019).

Recently, several WWTPs have implemented a combined activated carbon adsorption and ozonation treatment method as a cost-efficient process in Germany and Switzerland. Since reverse osmosis membranes are cost-intensive, this membrane filtration process also provides additional benefits concerning salinity and metal reduction. Reverse osmosis membranes are utilized in potable reuse schemes in the United States of America, Singapore, and Australia (Rizzo et al., 2019).

CONCLUSIONS

The increasing use of antibiotics in human medicine and animal husbandry has adverse impacts, and this contamination should be addressed as a global health and environmental issue. It is essential to ensure that antibiotic residues, ARB, and ARG, are satisfactorily eliminated from the environment, minimizing potential risks to human health.

From a practical point of view, recent advancements in wastewater treatments, especially utilizing AOPs, are primarily based on bench-scale experiments and pilot-scale in batch mode. However, for large-scale implementation in real systems, it is imperative to evaluate the design and application of combined treatment technologies. Additionally, other parameters such as process optimization, risk assessment, and pollution control should be monitored for comparison and further assessment as most current studies focus on evaluating and achieving successful degradation efficiency performance of antibiotics.

As a result, more comprehensive research is needed to reinforce the investigation of the mineralization of CECs using different AOPs-based combined treatment methods for large-scale applications. Furthermore, designing cost-effective and environmentally friendly treatments is critically important, serving as more efficient alternatives to currently available treatment technologies used in WWTPs that are still needed in the future.

REFERENCES

Berges, J., Moles, S., Ormad, M.P., Mosteo, R. and Gómez, J. 2021. Antibiotics removal from aquatic environments: adsorption of enrofloxacin, trimethoprim, sulfadiazine, and amoxicillin on vegetal powdered activated carbon. Environ. Sci. Pollut. Res. 28(7), 8442-8452.

Conco, T., Kumari, S., Awolusi, O.O., Allam, M., Ismail, A., Stenström, T.A. and Bux, F. 2022. Profiling of emerging pathogens, antibiotic resistance genes and mobile genetic elements in different biological wastewater treatment plants. J. Environ. Chem. Eng. 10(3), 107596.

Deniere, E., Alagappan, R.P., Langenhove, H.V., Hulle, S.V. and Demeestere, K. 2022. The ozone-activated peroxymonosulfate process (O_3/PMS) for removal of trace organic contaminants in natural and wastewater: Effect of the (in)organic matrix composition. Chem. Eng. J. 430, 133000.

Drane, K., Sheehan, M., Whelan, A., Ariel, E. and Kinobe, R. 2024. The Role of Wastewater Treatment Plants in Dissemination of Antibiotic Resistance: Source, Measurement, Removal and Risk Assessment. Antibiotics 13(7), 668.

Garrido-Cardenas, J.A., Esteban-García, B., Agüera, A., Sánchez-Pérez, J.A. and Manzano-Agugliaro, F. 2020. Wastewater Treatment by Advanced Oxidation Process and Their Worldwide Research Trends. International Journal of Environmental Research and Public Health 17(1), 170.

Godoy, M. and Sánchez, J. (2020) Antibiotic Materials in Healthcare. Kokkarachedu, V., Kanikireddy, V. and Sadiku, R. (eds), pp. 221-230, Academic Press.

Hutchings, M.I., Truman, A.W. and Wilkinson, B. 2019. Antibiotics: past, present and future. Current Opinion in Microbiology 51, 72-80.

Khmaissa, M., Zouari-Mechichi, H., Sciara, G., Record, E. and Mechichi, T. 2024. Pollution from livestock farming antibiotics an emerging environmental and human health concern: A review. Journal of Hazardous Materials Advances 13, 100410.

Li, Y., Wang, J., Lin, C., Lian, M., He, M., Liu, X. and Ouyang, W. 2024. Occurrence, removal efficiency, and emission of antibiotics in the sewage treatment plants of a low-urbanized basin in China and their impact on the receiving water. Sci. Total Environ. 921, 171134.

Lu, H., Feng, W. and Li, Q. 2022. Degradation Efficiency Analysis of Sulfadiazine in Water by Ozone/Persulfate Advanced Oxidation Process. Water 14(16), 2476.

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C. and Fatta-Kassinos, D. 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. Water Res. 47(3), 957-995.

Moles, S., Ormad, M.P., Gomez, J., Szpunar, J., Bolea, E. and Mosteo, R. 2024. Pilot plant approach combining photocatalysis and adsorption for antibiotics removal from slaughterhouse and urban wastewater treatment plant effluents. Environ. Technol. Innov. 34, 103586.

O'Neill, J. 2016. Tackling drug-resistant infections globally : final report and recommendations Review on Antimicrobial Resistance.

Rizzo, L., Malato, S., Antakyali, D., Beretsou, V.G., Đolić, M.B., Gernjak, W., Heath, E., Ivancev-Tumbas, I., Karaolia, P., Lado Ribeiro, A.R., Mascolo, G., McArdell, C.S., Schaar, H., Silva, A.M.T. and Fatta-Kassinos, D. 2019. Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. Sci. Total Environ. 655, 986-1008.

Rodríguez-Chueca, J., Varella della Giustina, S., Rocha, J., Fernandes, T., Pablos, C., Encinas, Á., Barceló, D., Rodríguez-Mozaz, S., Manaia, C.M. and Marugán, J. 2019. Assessment of full-scale tertiary wastewater treatment by UV-C based-AOPs: Removal or persistence of antibiotics and antibiotic resistance genes? Sci. Total Environ. 652, 1051-1061.

Wang, B., Xu, Z. and Dong, B. 2024. Occurrence, fate, and ecological risk of antibiotics in wastewater treatment plants in China: A review. J. Hazard. Mater. 469, 133925.

Wang, Y., Han, Y., Li, L., Liu, J. and Yan, X. 2022. Distribution, sources, and potential risks of antibiotic resistance genes in wastewater treatment plant: A review. Environ. Pollut. 310, 119870.

Yang, L., Wen, Q., Chen, Z., Duan, R. and Yang, P. 2019. Impacts of advanced treatment processes on elimination of antibiotic resistance genes in a municipal wastewater treatment plant. Frontiers of Environmental Science & Engineering 13(3), 32.

Yang, W., Li, J., Yao, Z. and Li, M. 2024. A review on the alternatives to antibiotics and the treatment of antibiotic pollution: Current development and future prospects. Sci. Total Environ. 926, 171757.

Zhang, T. and Li, B. 2011. Occurrence, Transformation, and Fate of Antibiotics in Municipal Wastewater Treatment Plants. Critical Reviews in Environmental Science and Technology 41(11), 951-998.

Zhang, X.Y., Liu, T.S. and Hu, J.Y. 2024a. Antibiotics removal and antimicrobial resistance control by ozone/peroxymonosulfate-biological activated carbon: A novel treatment process. Water Res. 261, 122069.

Zhang, Y., Hu, Y., Li, X., Gao, L., Wang, S., Jia, S., Shi, P. and Li, A. 2024b. Prevalence of antibiotics, antibiotic resistance genes, and their associations in municipal wastewater treatment plants along the Yangtze River basin, China. Environ. Pollut. 348, 123800.

Zhuang, Y., Ren, H., Geng, J., Zhang, Y., Zhang, Y., Ding, L. and Xu, K. 2015. Inactivation of antibiotic resistance genes in municipal wastewater by chlorination, ultraviolet, and ozonation disinfection. Environ. Sci. Pollut. Res. 22(9), 7037-7044.

PHOTOCATALYTIC DEGRADATION OF TEXTILE AZO IN AQUEOUS SOLUTION USING NEODYMIUM (III) OXIDE

Yeliz AKPINAR, Nazli TURKTEN, Yunus KARATAS*

Kirsehir Ahi Evran University, Faculty of Arts and Sciences, Department of Chemistry, 40100, Kirsehir, Turkiye.
Corresponding author e-mail: nazli.turkten@yahoo.com

ABSTRACT

A major water pollution problem today is related to the contamination of industrial effluent-induced textile dyes. Improper discharge of industrial wastewater can be the main source of water contamination and a potential threat to aquatic life and human health since it is highly resistant to conventional treatment methods. Photocatalysis arises as a promising alternative method to eliminate azo dyes. Rare earth metal oxides, particularly neodymium (III) oxide (Nd_2O_3) have emerged as catalysts in photocatalytic applications. In this study, Nd_2O_3 nanoparticles were characterized by FT-IR, SEM, and Raman spectroscopy. FT-IR spectroscopy revealed the characteristic bands related to the metal-oxygen groups. Reactive Red 194 (RR-194) is an azo dye containing an azo group as a chromophore, widely used in the textile industry to color cotton fabrics. The photocatalytic degradation efficiencies of RR-194 dye were compared under both UV and solar-simulated light (SI) conditions. Test conditions such as catalyst dose (0.25 g/L and 0.50 g/L) and initial RR-194 dye concentration (10 mg/L, 20 mg/L, 30 mg/L) were also investigated. Results revealed that the maximum decolorization rate of RR-194 was observed with the increased catalyst dosage and decreased initial dye concentration.

Keywords: Azo dye, decolorization, heterogeneous photocatalysis, neodymium (III) oxide, Reactive Red 194.

INTRODUCTION

Water pollution related to industrial growth, especially from the expansion of textile industries, presents an essential public concern. However, dyes do not fully bind to the fabric in dyeing, and the characteristics of discharged wastewater contain approximately 11-15 % of unbound dyes, salts, chemical variants, and non-biodegradable organic substances as textile effluents. Even low concentrations of dyes in textile wastewater can disrupt the ecosystem and cause notable harm to aquatic life on a larger scale. Therefore, efficient wastewater treatment is crucial to meet the required standards before discharging into the water environment. However, achieving a complete dye elimination treatment process that leads to minimal waste emission is a remarkable challenge due to the recalcitrant nature of synthetic dyes, mainly derived from coal and petroleum intermediates (Ahtasham Iqbal et al., 2024; Khan et al., 2024; Palak et al., 2024; Sofia et al., 2024).

Effluent treatment plants are widely accepted approaches for treating industry effluents, enabling further reuse in different processes. However, the selected method depends on the composition, characteristics, and concentration of effluent, and implementing a single process falls short of entirely degrading the dyes. Although biological systems are quite popular with an economic nature, they suffer from slow kinetics and insufficient degradation performance. Similarly, inferior efficient treatment techniques such as flocculation, filtration, and sedimentation cause secondary pollution (Ahtasham Iqbal et al., 2024; Khan et al., 2024; Palak et al., 2024).

Advanced oxidation processes (AOPs) refer to exceptional chemical oxidation processes via direct or indirect commonly used for a comprehensive range of dyes and persistent organic compounds that cannot degrade efficiently through conventional methods. AOPs can be classified as Fenton, ozonation, ultrasonic, electrochemical oxidation, H_2O_2/UV, and photocatalysis based on the generation of principle and oxidizing species. Among AOPS, photocatalysis is a superior and environmentally friendly treatment that produces highly reactive hydroxyl radicals upon exposure to light and operates under low operational costs. Metal oxides, particularly TiO_2 and ZnO have been extensively used as photocatalysts (Ahtasham Iqbal et al., 2024; Khader et al., 2024; Khan et al., 2024; Palak et al., 2024). Although Nd_2O_3, a rare earth compound has been applied in distinct fields, there are only limited photocatalytic studies. Some of the related studies reported so far are the photocatalytic removal efficiencies of methylene blue (Ahmed et al., 2022; Sokkalingam et al., 2024; Zinatloo-Ajabshir et al., 2017b), Rhodamine B (Mortazavi-Derazkola et al., 2015), Eriochrome black T (Zinatloo-Ajabshir et al., 2017c), Eosin Y (Zinatloo-Ajabshir et al., 2017a) in the presence of Nd_2O_3.

In the present study, the structural and morphological properties of Nd_2O_3 particles were identified using FT-IR, SEM, and Raman spectroscopy. Reactive Red 194 (RR-194) was chosen as an azo dye used in the textile industry, particularly cotton dyeing. The photocatalytic degradation of RR-194 under both UV and solar-simulated light (SI) conditions were tested. In addition, catalyst dose and initial dye concentration parameters were investigated.

MATERIAL AND METHOD

Neodymium (III) oxide (Nd_2O_3, analytical grade) was obtained from Sigma Aldrich and used without further treatment. All aqueous solutions were prepared with distilled water (conductivity 2 µS/cm at 25 °C). The chemical structure of RR-194 dye is given in Figure 1.

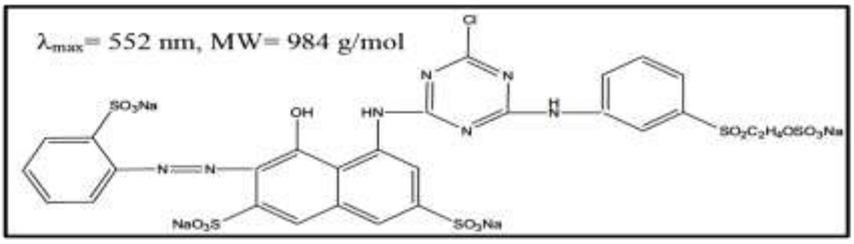


Figure 1. The chemical structure of RR-194.

FT-IR spectroscopy was employed using a Thermo Scientific Nicolet 6700 spectrometer equipped with an attenuated total reflection accessory. FT-IR measurement was carried out in 32 scans at a resolution of 4 cm^{-1} in the 4000–400 cm^{-1} range. Dispersive Raman spectroscopic measurement was performed on a Thermo Scientific DXR Raman Microscope with an Ar^+ laser power (10 mW at λ=532 nm) and a resolution of 4 cm^{-1}. SEM analysis was performed on FEI-Philips XL30 Scanning Electron Microscope with an accelerating voltage of 10 kV.

The photocatalytic activity tests were performed in a cylindrical Pyrex reaction vessel under UV and SI conditions and the reactor was irradiated from the top. A 125W black light fluorescent lamp ($\lambda =_{max}$ 365 nm) and an OSRAM Ultra-Vitalux lamp (300 W) were used as the UV and SI light sources respectively. The photocatalytic experiments were tested using Nd_2O_3 dose (0.25 g/L and 0.50 g/L) and initial RR-194 dye concentration (10 mg/L, 20 mg/L, 30 mg/L) without pH adjustment. The photocatalyst was dispersed in 50 mL of RR-194 solution and the irritated solution was immediately filtered through 0.22 μm cellulose acetate filters. The absorbance values of the solutions were performed on a Thermo Scientific Genesys 10S double-beam spectrophotometer using 1 cm quartz cells.

RESULTS AND DISCUSSION

FT-IR spectrum of the Nd_2O_3 specimen is presented in Figure 2 (a). A strong band observed at 3606 cm^{-1} corresponded to the O-H stretching of physisorbed water molecules (Zinatloo-Ajabshir et al., 2017c). The characteristic metal-oxygen bands at 661 cm^{-1} and 459 cm^{-1} were attributed to the Nd-O stretching vibrations (Sokkalingam et al., 2024). The appeared band at 1473 cm^{-1} could belong to C=O implying atmospheric CO_2 (Ahmed et al., 2022). Raman spectrum of the Nd_2O_3 is represented in Figure 2 (b). The four characteristic Raman modes of Nd_2O_3 at 183 cm^{-1}, 296 cm^{-1}, 360 cm^{-1}, and 472 cm^{-1} were observed (Sokkalingam et al., 2024). SEM images of Nd_2O_3 exhibit various polyhedral-shaped particles with aggregation (Figure 3).

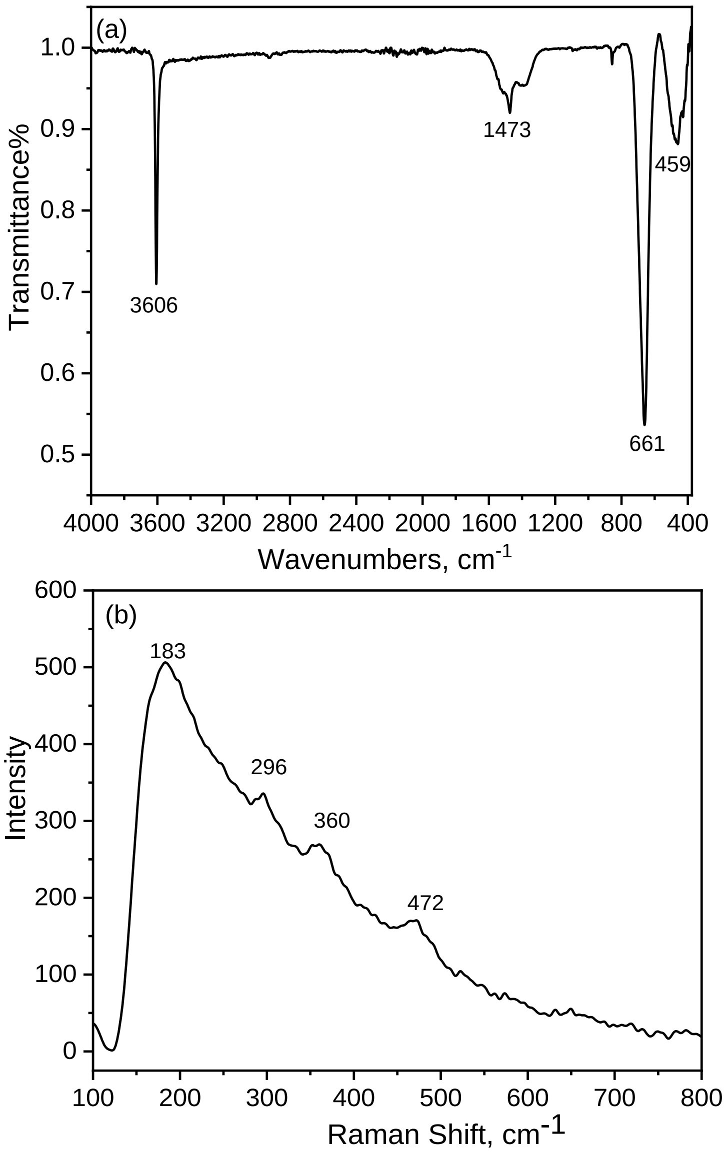


Figure 2. (a) FT-IR spectrum and (b) Raman spectrum of Nd_2O_3 specimen.

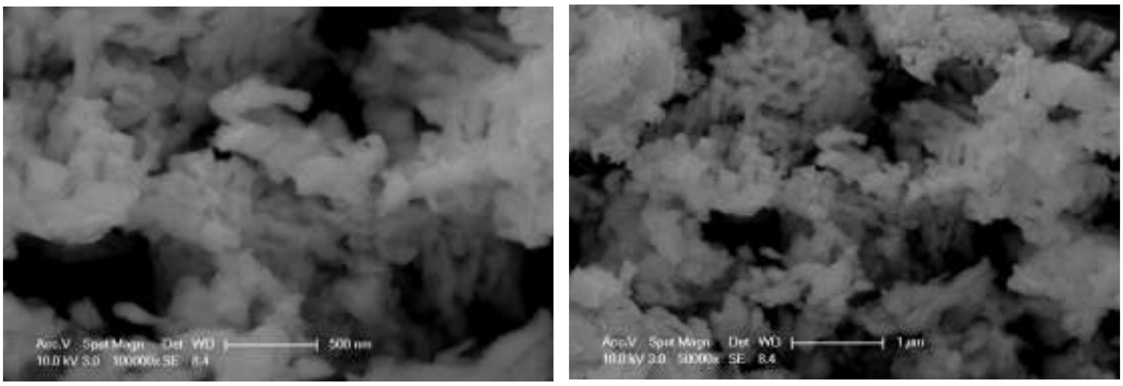


Figure 3. SEM images (left) x100000 and (right) x50000 of Nd_2O_3 specimen.

The degree of RR-194 decolorization in the presence of Nd_2O_3 was calculated by the following equation (1).

Decolorization, %= $((A_{,o} - A)/A_o))$ x100 (1)

where,

$A_{,o}$ = initial absorbance of RR-194 and A = absorbance of RR-194 at irradiation time t.

The degree of RR-194 decolorization was examined upon doses (0.25 g/L and 0.50 g/L) of Nd_2O_3 at 10 mg/L initial concentration of RR-194 dye under 300 min irradiation. To determine the effect of the light source, the experiments were tested under both UV and SI conditions (Figure 4 (a)). It was found that SI experiments revealed higher removal percentages than UV light conditions.

As catalyst concentration increased to 0.50 g/L, the removal percentages of RR-194 at 300 min irradiation were almost doubled (Figure 4 (b)). The highest removal efficiency of RR-194 in the presence of 0.50 g/L Nd_2O_3 under 300 min SI irradiation time was 46%. The reason could be the enhancement of photons and adsorbed dye molecules.

The initial RR-194 dye concentration effect at three different concentrations (10 mg/L, 20 mg/L, and 30 mg/L) was performed under UV light for 60 min in the presence of Nd_2O_3 (0.25 g/L and 0.50 g/L) (Figure 4 (c)). The removal efficiency decreased with increasing initial RR-194 concentration up to 0.50 g/L dose of Nd_2O_3 because the increased dye concentration may affect the light penetration into the reaction solution, leading to a low catalytic performance at higher dye concentrations. However, the removal values remained almost unchanged at higher concentrations (20 mg/L and 30 mg/L) of RR-194 with 0.25 g/L catalyst dose.

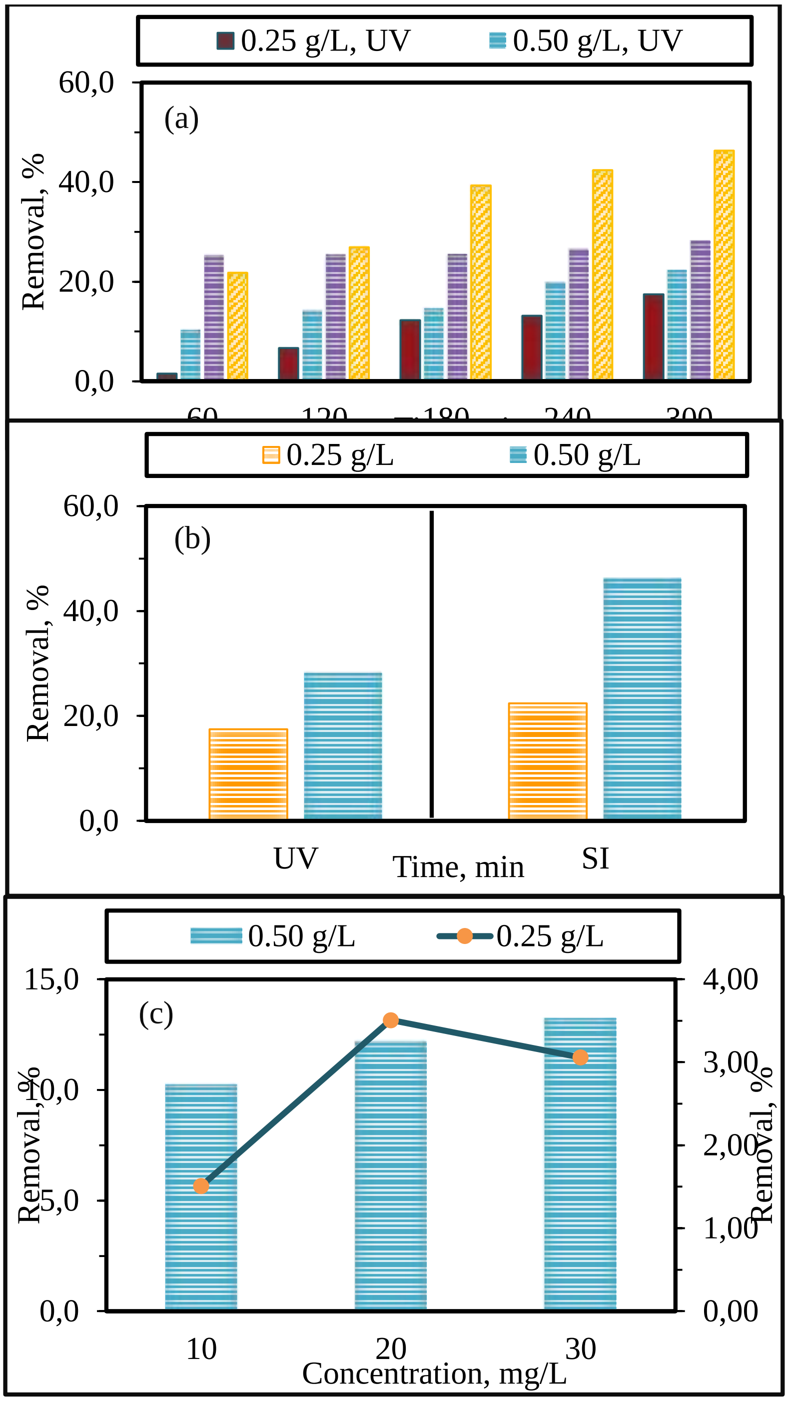


Figure 4. (a) Removal efficiencies of RR-194, (b) Dose effect of Nd_2O_3 specimen, and (c) Initial RR-194 dye concentration effect.

CONCLUSIONS

The decolorization performances of RR-194 in the presence of Nd_2O_3 specimen under both UV and SI systems was investigated. The removal efficiencies of RR-194 under the SI system were significantly higher compared to UV light conditions. The result indicated that a higher dose of Nd_2O_3 (0.50 g/L) was favorable. Additionally, a higher RR-194 dye concentration caused a decrease in light penetration. The structural properties of Nd_2O_3 were determined using FT-IR and Raman spectroscopy techniques. FT-IR confirmed the evidence of characteristic bands of Nd_2O_3 related to the metal-oxygen groups. SEM images revealed a variety of polyhedral-shaped particles with agglomeration.

REFERENCES

Ahmed, A.A., Rizvi, Z.R., Shahzad, H. and Farrukh, M.A. 2022. Neodymium oxide nanoparticles synthesis using phytochemicals of leaf extracts of different plants as reducing and capping agents: Growth mechanism, optical, structural and catalytic properties. J. Chin. Chem. Soc. 69(3), 462-475.

Ahtasham Iqbal, M., Akram, S., khalid, S., Lal, B., Hassan, S.U., Ashraf, R., Kezembayeva, G., Mushtaq, M., Chinibayeva, N. and Hosseini-Bandegharaei, A. 2024. Advanced photocatalysis as a viable and sustainable wastewater treatment process: A comprehensive review. Environ. Res. 253, 118947.

Khader, E.H., Muslim, S.A., Saady, N.M.C., Ali, N.S., Salih, I.K., Mohammed, T.J., Albayati, T.M. and Zendehboudi, S. 2024. Recent advances in photocatalytic advanced oxidation processes for organic compound degradation: A review. Desalination and Water Treatment 318, 100384.

Khan, S., Noor, T., Iqbal, N. and Yaqoob, L. 2024. Photocatalytic Dye Degradation from Textile Wastewater: A Review. ACS Omega 9(20), 21751-21767.

Mortazavi-Derazkola, S., Zinatloo-Ajabshir, S. and Salavati-Niasari, M. 2015. Preparation and characterization of Nd_2O_3 nanostructures via a new facile solvent-less route. J. Mater. Sci.: Mater. Electron. 26(8), 5658-5667.

Palak, B., Kumari, A. and Parmar, M.S. 2024. Textile Wastewater Treatment through AOPs with Special Focus on Fixed-Bed Approach: A Review. Journal of Water Chemistry and Technology 46(3), 292-301.

Sofia, D.R., Hanam, E.S., Sunardi, S., Sumiarsa, D. and Joni, I.M. 2024. Hydroxyl Radical-Based Advanced Oxidation Processes of Red Reactive Dyes by Ultrafine Bubbles Method. Water 16(12), 1678.

Sokkalingam, R., Devaraj Raja, G., Panghal, A., Roy, S.S., Sankaran, E.M., Anbalagan, B. and Sonachalam, A. 2024. Enhancing photocatalytic dye degradation efficiency through the utilization of shock wave effects on neodymium oxide (Nd_2O_3). J. Mol. Struct. 1318, 139215.

Zinatloo-Ajabshir, S., Mortazavi-Derazkola, S. and Salavati- Niasari, M. 2017a. Sonochemical synthesis, characterization and photodegradation of organic pollutant over Nd_2O_3 nanostructures prepared via a new simple route. Sep. Purif. Technol. 178, 138-146.

Zinatloo-Ajabshir, S., Mortazavi-Derazkola, S. and Salavati-Niasari, M. 2017b. Nd_2O_3 nanostructures: Simple synthesis, characterization and its photocatalytic degradation of methylene blue. J. Mol. Liq. 234, 430-436.

Zinatloo-Ajabshir, S., Mortazavi-Derazkola, S. and Salavati-Niasari, M. 2017c. Schiff-base hydrothermal synthesis and characterization of Nd_2O_3 nanostructures for effective photocatalytic degradation of eriochrome black T dye as water contaminant. J. Mater. Sci.: Mater. Electron. 28(23), 17849-17859.

RISK MANAGEMENT AND SUSTAINABILITY IN WATERMELON PRODUCTION IN KARATAS DISTRICT OF ADANA PROVINCE

Handan AKCAOZ *Remziye SUNA* *Muhammet Veli Ince*

Akdeniz University, Faculty of Agriculture, Department of Agricultural Economics,

07070 Antalya, Türkiye

hvurus@akdeniz.edu.tr, remziyesuna@akdeniz.edu.tr, muhammetveliince@icloud.com

ABSTRACT

In this study, risk management and sustainability issues in agricultural enterprises producing watermelon in Karatas district of Adana province were examined. In the agricultural enterprises within the scope of the study, issues such as demographic characteristics, risk sources encountered in agriculture, risk management strategies, farmers' reasons for agricultural production, thoughts of continuing agricultural production in the future, and information sources for agricultural production were investigated. In the study, data obtained from primary and secondary sources were used. The primary source of the study was the data obtained from the surveys applied to 32 farmers producing watermelon in the 2022 production period in Karatas district of Adana province. Similar studies conducted nationally and internationally, reports and records of relevant institutions and organizations are the secondary sources of the study. According to the results obtained from the study; changes in input costs, changes in interest rates, changes in product yields, lack of adequate support for basic inputs such as fertilizers, pesticides, irrigation and diesel fuel, and the high difference between consumer and producer prices are important risk sources affecting watermelon production. According to the data obtained from the surveys applied to farmers in farms producing watermelon; planning expenditures, increasing solvency, planning borrowing, and preventing excessive use of existing resources have been identified as important strategies to manage risk.

Keywords: Risk Management, Risk Resources, Sustainability, Watermelon, Adana

INTRODUCTION

Every year, 101 million tons of watermelon are produced in approximately 3 million hectares of land in the world. The amount of watermelon exported in the world is 4.5 million tons. The total amount of world watermelon imports is 4.2 million tons. In watermelon production, China, Türkiye and India respectively; Spain, Mexico, Italy in watermelon exports; The USA, Germany and Canada are the leading countries in watermelon imports.

Agriculture is one of the leading sectors in Turkey, which has favorable geographical conditions and climate, a rich soil structure and biological diversity. Watermelon is a product of great economic importance in Turkish agriculture. According to the "Plant Product Balance Tables" prepared by TÜİK (2024), regarding watermelon production in the 2022/2023 period; production amount 3394783 tons, production losses 91659 tons, supply/use 3348971 tons, usable production 3303124 tons, imports 45847 tons, domestic use 3204983 tons, fresh consumption 2884485 tons, exports 143988, per capita consumption 33.8 kg., degree of sufficiency 103.1% values are given. According to these data, Türkiye is a self-sufficient country in watermelon production. The top countries in Turkey's watermelon exports by country in the 2021/22 production year are Bosnia (14.3%), Russia (13.8%), Georgia (10.3%), Iraq (8.4%), Syria (7.8%) and other countries (45.5%). Iran ranks first in watermelon imports. In addition, the majority of watermelon production is consumed fresh (TUIK, 2024). In Turkey, the regions where watermelon is produced the most are; Aegean, Mediterranean, Southeastern Anatolia and Marmara regions. On a provincial basis, the highest production is made in Adana (19.9%). In watermelon production, Adana province is followed by Antalya (13%), Mersin (7.3%), Bursa (5.6%), Adıyaman (3.7%), Diyarbakır (3.5%), Manisa (3.5%), Samsun (3.2%) and other provinces follow (Kaya, 2022).

In a study conducted in Antalya, the problems faced by producers in watermelon production were determined as high input prices, fight against diseases and pests, climatic conditions, insufficient supports, problems in irrigation, labor, labor supply and cost. The problems of watermelon producers in the marketing process vary depending on the producer and business conditions, but are expressed as the lack of number and variety of buyers, excessive fluctuation and uncertainty of prices, low prices, long payment terms, lack of bargaining opportunities, and product losses, respectively (Buyukkalay, 2019).

In the agricultural sector, whose development is important for Turkey, production is exposed to natural risks that are not under the control of the producer, despite today's developing technology. In this respect, agriculture is described as an open-top factory. Developed countries have been pursuing protectionist policies under the name of 'Risk Management Programs in Agriculture' for years due to the natural, economic, social, technological and personal risks they face. With these programs, after the productivity losses resulting from natural risks that threaten agricultural production, they also take the income losses resulting from economic risks from the agricultural sector and transfer them to insurance systems (Gungor, 2006).

Although farmers manage only part of the production process, risk management at the business level is extremely important. Because failure to cope with the risks inherent in agricultural production will result in loss of income and livelihood for millions of people in developing countries. Additionally, all other decision makers in the agricultural supply chain must face risks in their decision-making processes. However, farmers in developing countries have insufficient capacity to manage agricultural risk at the enterprise level (Akcaoz et al., 2024).

Since agricultural production is open to natural conditions; adverse climatic conditions such as floods, drought, hail, frost, diseases and pests often cause devastating damage to the producer's income. These devastating damages are the most important factors that threaten the continuity of agricultural production. In order to prevent these risks that prevent the continuity of agricultural production, technical protection methods are primarily used. Farmers who want to protect themselves from the risks affecting agricultural production, make a better living and improve their living standards diversify their products and work in various activities outside the business. However, these measures taken by farmers to protect themselves from various risks cannot protect the income of the farmer family and may cause the farmer's income to decrease due to the expenses he makes (Ozsayin, 2005). There is an extensive literature examining farmers' perceptions, attitudes and behaviors towards risk sources and risk management strategies in agricultural enterprises nationally and internationally. Some of these studies on plant production are; Pellegrino (1999), Nicol et al. (2007), Ali and Kapoor (2008), Akcaoz et al. (2009), Adanacioglu and Olgun (2010), Sookhtanlo and Sarani (2011), Cetin and Esengun (2012), Almadani (2014), Agir et al. (2015), Bagheri and Fami (2016), Iqbal et al. (2016), Alp (2017), Rizwan et al. (2017), Usman et al. (2017), Akhtar et al. (2018), Bal (2018), Iqbal et al. (2018), Akhtar et al. (2019), Hayran (2019), Ucar and Engindeniz (2019), Ahmed et al. (2020), Hakorimana and Akcaoz (2020), Ozer and Tumer (2020), Agir and Erdem (2022).

In this study, risk management and sustainability issues were examined in agricultural enterprises producing watermelon in Karataş district of Adana province. In the agricultural enterprises within the scope of the study; demographic characteristics, risk sources encountered in agriculture, risk management strategies, farmers' reasons for agricultural production, thoughts of continuing agricultural production in the future, information sources for agricultural production, etc. topics have been researched.

MATERIALS AND METHODS

In the study, data obtained from primary and secondary sources were used. The primary source of the study is the data obtained from the surveys to be applied to farmers producing watermelon in Karataş district of Adana province. A survey was applied to 32 farmers producing watermelon in Karataş district of Adana province in the 2022 production period. The farmers who were surveyed were selected randomly and a face-to-face survey was conducted. In the farms within the scope of the survey, demographic characteristics, risks encountered in watermelon production, strategies that can be applied against risks, farmers' opinions about sustainability in watermelon production, information sources regarding agricultural activities, etc. Topics are included. The data obtained in this survey application were summarized and interpreted in tables. Likert scale was also used while obtaining primary data. Survey data were analyzed in the SPSS package program.

Similar studies conducted nationally and internationally, reports and records of relevant institutions and organizations are secondary sources.

RESEARCH FINDINGS

Demographic characteristics

Demographic characteristics were also included in the survey form applied to farmers in farms producing watermelon in Karataş district of Adana province. In this context, the gender, age, education level of family members, average family size, farmers' experience in agricultural production and watermelon production, working conditions in the farm and outside the farm were investigated (Table 1). The total number of family members in the farms within the scope of the research is 118, of which 53.39% are male and 46.61% are female. In the farms examined, 56.78% of the family members are in the 15-49 age group, 22.88% are over 50 years old, 11.02% are in the 7-14 age group and 9.32% are in the 0-6 age group. 56.25% of the surveyed farmers are in the 15-49 age group and 43.75% are in the over 50 age group. 53.39% of family members in farms are primary school graduates, and this rate is 62.5% in farmers. It was determined that the average experience period of the farmers in the research in agricultural production was 31.81 years, and the average experience period in watermelon production was 25.46 years. According to the findings of the research, 38.92% of family members work in their own businesses. The rate of family members working in non-agricultural jobs is 9.32%.

Table 1. Demographic Characteristics of the Farms Examined

	Person	**%**
Gender		
Male	63	53.39
Female	55	46.61
Total	***118***	***100.00***
Age Groups of Family Members		
0 – 6	11	9.32
7 – 14	13	11.02
15 – 49	67	56.78
50 +	27	22.88

Total	***118***	***100.00***
Age Groups of Farmers		
7 – 14	-	-
15 – 49	18	56.25
50 +	14	43.75
Total	***32***	***100.00***
Educational Status of the Family Members		
Illiterate*	11	9.32
Literate	12	10.17
Primary School Graduate	38	32.20
Secondary School Graduate	25	21.19
High School Graduate	20	16.95
Graduated from a University	12	10.17
Total	***118***	***100.00***
Educational Status of Farmers		
Literate	2	6.25
Primary School Graduate	14	43.75
Secondary School Graduate	6	18.75
High School Graduate	9	28.12
Graduated from a University	1	3.13
Total	***32***	***100.00***
Average Family Size	***3.69***	
Experience Period in Agricultural Production (Year)		
Minimum	4	
Maximum	68	
Average	31.81	
Experience Period in Watermelon Production (Year)		
Minimum	4	
Maximum	34	
Average	25.46	
Working Status in Own Farm		
Yes	46	38.92
No	72	61.02
Total	***118***	***100.00***
Working Status in Agricultural Works Outside the Own Farm		
Yes	1	0.85
No	117	99.15
Total	***118***	***100.00***
Working Status in Non-Agricultural Works		
Yes	11	9.32
No	107	90.68
Total	***118***	***100.00***

*Individuals who are not of school age

Land Characteristics in the Examined Farms

The total land width in the 32 enterprises within the scope of the research is 11591 decares and the average land width per farm is 362 decares. The total number of parcels in the farms is 78 and the average number of parcels per farm is 2.44. In the farms examined in the study, data regarding the ownership status of the lands, topographic condition, soil structure, soil fertility and land type were investigated. Considering the number of parcels of the farms, it is seen that 11.54% consists of rented land and 88.46% consists of freehold land. When the total land width is taken into consideration, it is seen that 13.04% consists of rented land and 86.96% consists of freehold land. In the farms within the scope of the research, 92.66% of the total land is flat and 7.34% has a slightly rugged topographic structure. Of the total land assets of the examined farms, 91.77% consists of high and 8.23% medium productive land. The soil structure of the total land assets in the farms is sandy and the land type consists of irrigated fields (Table 2).

Table 2. Land Characteristics in the Examined Farms

	Number of Parcels		Size of Land	
	Number	**%**	**Decare**	**%**
Land Ownership Status				
Own Land	69	88.46	10079	86.96
Rented Land	9	11.54	1512	13.04
Total	***78***	***100.00***	***11591***	***100.00***
Topographic Structure				
Flat land	69	88.46	10740	92.66
Slightly Rough Terrain	9	11.54	851	7.34
Total	***78***	***100.00***	***11591***	***100.00***
Soil Structure				
Sandy Soil	78	100.00	11591	100.00
Total	***78***	***100.00***	***11591***	***100.00***
Soil Fertility				
High	69	88.46	10637	91.77
Middle	9	11.54	954	8.23
Total	***78***	***100.00***	***11591***	***100.00***
Land Type				
Irrigated Field Land	78	100.00	11591	100.00
Total	***78***	***100.00***	***11591***	***100.00***

Risk Sources in Farms

Sources of Production Risk

Crop and livestock production performance depends on biological processes that are affected by weather conditions, diseases and pests. Insufficient rainfall or drought can cause low yields. Hail or heavy rains can damage or completely destroy crops. Pest or disease outbreaks can also cause major productivity losses in crop production and livestock. In agricultural production, when farmers plant seeds and fertilize their fields, they do not know how much rain will fall, whether there will be hail or a storm. They cannot predict whether there will be a problem with pests or diseases. But they still have to continue their production activities.

In the research, the sources of production risks encountered in businesses producing watermelon are given in Table 3. Farmers' evaluations of production risk sources in the examined farms were determined on a Likert scale. Accordingly, some of the sources of production risk that farmers express as very important in watermelon production are; changes in crop yields, low productivity due to plant pests, lack of information about the production method, frost, excessive rainfall, insufficient rainfall, inadequacy of agricultural tools and machinery, changes in climate conditions, low productivity due to plant diseases, crop damage due to floods, inadequacy of analysis laboratories, difficulties in finding foreign labor and crop losses during harvest (Table 3).

Table 3. Sources of Production Risk in the Examined Farms

Production risk	**Mean**	**Std. Deviation**	**Scale (%)**					
			1	**2**	**3**	**4**	**5**	**Total**
Changes in crop yields	1.00	0.00	100.0	-	-	-	-	100.0
Low productivity due to plant pests	1.00	0.00	100.0	-	-	-	-	100.0
Lack of information about the production method	1.00	0.00	100.0	-	-	-	-	100.0
Frost	1.00	0.00	100.0	-	-	-	-	100.0
Excessive rainfall	1.00	0.00	100.0	-	-	-	-	100.0
Insufficient rainfall	1.00	0.00	100.0	-	-	-	-	100.0
Inadequacy of agricultural tools and machinery	1.00	0.00	100.0	-	-	-	-	100.0
Lack of technical knowledge of farmers	1.00	0.00	100.0					100.0
Changes in climate conditions	1.03	0.17	96.9	3.1	-	-	-	100.0
Low productivity due to plant diseases	1.03	0.17	96.9	3.1	-	-	-	100.0
Crop damage due to floods	1.12	0.70	96.9	-	-	-	3.1	100.0
Inadequacy of analysis laboratories	1.25	0.87	90.6	3.1	-	3.1	3.1	100.0
Difficulties in finding foreign labor	1.31	0.78	78.1	18.8	-	-	3.1	100.0
Crop losses during harvest	1.50	0.62	56.3	37.5	6.3	-	-	100.0
Changes in land prices	1.68	0.89	53.1	31.3	9.4	6.3	-	100.0
No division of labor	2.15	1.05	31.3	34.4	25.0	6.3	3.1	100.0
Crop damage due to fire	2.28	1.46	46.9	15.6	9.4	18.8	9.4	100.0
Theft	2.53	1.01	15.6	37.5	25.0	21.9	-	100.0
Landslide	4.03	1.59	15.6	9.4	-	6.3	68.8	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

Sources of Financing Risk

Financing risk occurs when money is borrowed to finance a farming business. This risk can be caused by uncertainty in future interest rates, the lender's willingness to provide funds when needed, and the farmer's ability to generate the income necessary to repay the loan. Small farmers who borrow money at high interest rates may have difficulty paying their debts. Lower than expected prices combined with low yields can make debt repayment difficult and even lead to the sale of the business. The opinions of farmers about financing risk sources in the examined farms are given in Table 4. According to the farmers surveyed in the study, some of the most important sources of financing risks encountered in watermelon production are; changes in interest rates, changes in input costs, debt structure, debt status, inadequacy of financing, capacity and availability of credit resources, financing without equity capital, insufficiency in farm capital and inflation.

Table 4. Sources of Financing Risk in the Examined Farms

Financing risk	Mean	Std. Deviation	Scale (%)					
			1	2	3	4	5	Total
Changes in interest rates	1.00	0.00	100.0	-	-	-	-	100.0
Changes in input costs	1.00	0.00	100.0	-	-	-	-	100.0
Debt structure (Maturity of debt, source of debt)	1.03	0.17	96.9	3.1	-	-	-	100.0
Debt status (Debt amount)	1.03	0.17	96.9	3.1	-	-	-	100.0
Inadequacy of financing	1.03	0.17	96.9	3.1	-	-	-	100.0
Capacity and availability of credit resources	1.03	0.17	96.9	3.1	-	-	-	100.0
Financing without equity capital	1.06	0.24	93.8	6.3	-	-	-	100.0
Insufficiency in farm capital	1.09	0.39	93.8	3.1	3.1	-	-	100.0
Inflation	1.40	0.97	84.4	-	6.3	9.4	-	100.0
Failure to keep farm records	2.28	1.46	46.9	15.6	9.4	18.8	9.4	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

Sources of Marketing and Price Risk

Market and price risk relates to the volatility of prices and uncertainty of markets for agricultural products. Since agricultural product prices vary significantly from year to year, they affect the supply of goods and therefore the production decisions of the farmer. Demand is affected by factors such as consumer incomes, export policies, the state of the general economy, and the supply of competing products. In addition, some price movements show seasonality. Costs are another source of price risk. Input prices vary less than product prices. However, there is also uncertainty in input prices. Since the production cost per unit depends on yield and prices, its variability is quite high. In short, input and product prices in agricultural production are the elements that cause marketing and price risks.

The opinions of farmers regarding marketing and price risk in the farms examined in the study were determined with the Likert scale and the obtained data are summarized in Table 5. Among the marketing and price risks expressed by farmers in the watermelon producing farms in the research area as very important are; changes in the prices of the crops, lack of information about marketing and sales, lack of market guarantee for the crops, problems encountered in preservation (storage), inability to grow products of suitable variety and quality for the market, problems encountered in transportation and lack of infrastructure in wholesalers.

Table 5. Sources of Marketing and Price Risk in the Examined Farms

Marketing and Price Risk	Mean	Std. Deviation	Scale (%)					
			1	2	3	4	5	Total
Changes in the prices of the crops	1.00	0.00	100.0	-	-	-	-	100.0
Lack of information about marketing and sales	1.00	0.00	100.0	-	-	-	-	100.0
Lack of market guarantee for the crops	1.00	0.00	100.0	-	-	-	-	100.0
Problems encountered in preservation (storage)	1.25	0.57	81.3	12.5	6.3	-	-	100.0
Inability to grow products of suitable variety and quality for the market	1.28	0.92	90.7	-	3.1	3.1	3.1	100.0
Problems encountered in transportation	1.34	0.55	68.8	28.1	3.1	-	-	100.0
lack of infrastructure in wholesalers	1.34	0.97	87.5	-	6.3	3.1	3.1	100.0
Small farm scale	1.81	0.78	37.5	46.9	12.5	3.1	-	100.0
Inadequacies in producer organization	1.97	0.99	43.8	21.9	28.1	6.3	-	100.0
Distance to sales location	2.59	1.16	21.9	25.0	28.1	21.9	3.1	100.0
Problems encountered in packaging	4.09	1.20	6.3	3.1	18.8	18.8	53.2	100.0
Insufficiency of cold storage	4.25	1.11	3.1	9.4	3.1	28.1	56.3	100.0
No contract production	5.00	0.00	-	-	-	-	100.0	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

Personal Risk Sources (Human Originated Risks)

Human-caused risk refers to agribusiness risks resulting from illness or death and the personal situation of the farming family. Accidents, illnesses and death can negatively impact the performance of a farming business. It is common in many countries for the workforce to move away from rural areas. Migration may cause labor shortages in agricultural production activities. Political and social unrest may also limit labor availability. Farmers cannot be absolutely sure that they will be able to find the labor force required for crop and animal production activities in sufficient numbers at the required time. As agricultural enterprises grow, technology advances, and other risks increase, human-related risks become more important. Farmers are more knowledgeable and experienced in production-related issues than in risk-related issues. Human-related risks can be examined in three groups: the farmer and his family, the person managing the business and the employees of the business.

Farmers' opinions regarding personal risk sources in the farms within the scope of the research are given in Table 6. According to farmers, although the health/disability/death status of the business owner or worker is very important among the personal risk sources, work accidents occurring in the farm and problems/disputes in family relations are not important.

Table 6. Personal Risk Sources in the Examined Farms

Personal Risk	Mean	Std. Deviation	Scale (%)					
			1	2	3	4	5	Total
The health/disability/death status of the business owner or worker	1.40	0.66	68.8	21.9	9.4	-	-	100.0
Insufficiency of family workforce	2.46	0.94	9.4	56.3	12.5	21.9	-	100.0
Work accidents occurring in the farm	3.65	0.93	-	12.5	28.1	40.6	18.8	100.0
Problems/disputes in family relations	3.65	1.09	-	25.0	6.3	46.9	21.9	100.0

Scale * 1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important

Legal, Institutional and Political Risk Resources

Institutional risk refers to unforeseen changes in service delivery from institutions that support farming. Such institutions can be both formal and informal and may include banks, cooperatives, marketing organizations, input dealers and government agencies. Some of the institutional risk arises from the uncertainty of government policy affecting agriculture, such as price support and subsidies. The risks faced by farmers are often a result of decisions made by policy makers and managers. Subsidies, food quality regulations for export products, and the level of price or income support payments are examples of decisions made by the government that can have a major impact on agribusiness.

In the study, institutional and political risks were also evaluated within the scope of risk sources encountered in agricultural production. Farmers' opinions regarding institutional and political risk sources in the examined enterprises are given in Table 7. Accordingly, changes in the economic situation of the country, lack or insufficient government support, and changes in the general and agricultural policies implemented by the government were evaluated as very important for farmers.

Table 7. Legal/Institutional and Political Risk Sources in the Examined Farms

Legal, Institutional, Political Risk	Mean	Std. Deviation	Scale (%)					
			1	2	3	4	5	Total
Changes in the economic situation of the country	1.00	0.00	100.0	-	-	-	-	100.0
Lack or insufficient government support	1.00	0.00	100.0	-	-	-	-	100.0
Shanges in the general and agricultural policies implemented by the government (General and agricultural policy)	1.00	0.00	100.0	-	-	-	-	100.0

Scale * 1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important

Risk Management Strategies in Farms

Farmers have to find ways to cope with risk and protect themselves from decisions made today without knowing what will happen tomorrow. Risk management strategies are used to reduce the likelihood of a “bad” outcome occurring. Farmers trying to manage risk must identify possible sources of risk, recognize possible outcomes, decide on available alternative strategies, evaluate the consequences of each possible outcome, and evaluate the changes between the cost of risk and the gains to be achieved (Kahan, 2008).

Production Risk Management Strategies

Production risk arises from uncertainty regarding factors that affect the quantity and quality of agricultural products (e.g. weather, disease, pests, etc.). It also occurs with the use of new technologies. Various strategies can be used to reduce production risk. Some of those; risk-reducing inputs, risk-reducing technologies, diversification, insurance, non-business income, flexibility, etc. can be expressed as.

Farmers' opinions about the production risk management strategies that can be applied against the production risks encountered in the watermelon producing farms within the scope of the research are given in Table 8. Among the production risk management strategies, producing at the lowest cost possible, increasing supports for agricultural inputs, obtaining crop insurance, combating diseases with drugs, making differentiation in the farm, diversifying the farm and enlarging the farm land have been determined as very important.

Although land consolidation and regional production planning are institutional and political risk management tools, according to farmers, they are considered among the production risk management strategies and are expressed as very important. Having employees insured was stated as a production risk management strategy, but it was evaluated by farmers as not important in managing production risk.

Table 8. Production Risk Management Strategies in the Examined Farms

Production Risk Management Strategies	Mean	Std. Deviation	Scale (%)					
			1	2	3	4	5	Total
Producing at the lowest cost possible	1.00	0.00	100.0	-	-	-	-	100.0
Increasing supports for agricultural inputs	1.00	0.00	100.0	-	-	-	-	100.0
Obtaining crop insurance	1.00	0.00	100.0	-	-	-	-	100.0
Land consolidation	1.00	0.00	100.0	-	-	-	-	100.0
Regional production planning	1.00	0.00	100.0	-	-	-	-	100.0
Combating diseases with drugs	1.03	0.17	96.9	3.1	-	-	-	100.0
Differentiating the farm (including more than one product)	1.03	0.17	96.9	3.1	-	-	-	100.0
Diversifying the farm (include more than one type)	1.03	0.17	96.9	3.1	-	-	-	100.0
Enlarging the farm land	1.43	0.84	75.0	9.4	12.5	3.1	-	100.0
Working with an agricultural consultant	1.71	0.77	43.8	43.8	9.4	3.1	-	100.0
Reducing business land	2.50	1.45	40.6	9.4	18.8	21.9	9.4	100.0
Family members working outside the farm	3.31	1.46	15.6	18.8	12.5	25.0	28.1	100.0
Business owner working outside the business	3.62	1.23	3.1	25.0	6.3	37.5	28.1	100.0
Employees are insured	4.90	0.39	-	-	3.1	3.1	93.8	100.0
Getting greenhouse insurance	5.00	0.00	-	-	-	0.00	100.00	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

Financing Risk Management Strategies

Financial risk occurs when money is borrowed to finance the business. This risk is caused by uncertainty about future interest rates and repayment schedules, changes in loan collateral, and the farm's ability to generate the cash flow needed to repay the loan. Issues to be considered in financial risk management; credit availability and cost and repayment schedule, the farmer's liquidity or ability to generate cash flow, the farmer's ability to maintain and increase capital.

Financing risk management strategies were also investigated in the farms included in the study, and the findings are given in Table 9. According to the farmers surveyed in the study, financing risk management strategies such as planning expenses, reducing debt, increasing solvency, saving, finding capital resources, regulating the excessive use of existing resources, making financial analyzes and making non-farm investments are very important.

Table 9. Financing Risk Management Strategies in the Examined Farms

Financing Risk Management Strategies	Mean	Std. Deviation	Scale (%)					
			1	2	3	4	5	Total
Planning expenses	1.00	0.00	100.0	-	-	-	-	100.0
Reducing debt	1.00	0.00	100.0	-	-	-	-	100.0
Increasing solvency	1.00	0.00	100.0	-	-	-	-	100.0
Saving	1.00	0.00	100.0	-	-	-	-	100.0
Finding capital resources	1.00	0.00	100.0	-	-	-	-	100.0
Regulating the excessive use of existing resources	1.09	0.39	93.8	3.1	3.1	-	-	100.0
Making financial analyzes	1.09	0.39	93.8	3.1	3.1	-	-	100.0
Making non-farm investments	1.25	0.67	84.4	9.4	3.1	3.1	-	100.0
Keeping business records regularly	2.09	1.37	53.1	12.5	12.5	15.6	6.3	100.0
Debt management should be carried out by experts (Accountant, Agricultural Engineer, etc.)	2.68	0.89	3.1	50.0	21.9	25.0	-	100.0
Lowering the family's standard of living	2.96	0.96	-	40.6	28.1	25.0	6.3	100.0
Sell capital assets	5.00	0.00	100.0	-	-	-	-	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

Marketing and Price Risk Management Strategies

Marketing risk arises due to the variability in product prices and the uncertainty of future market prices that the farmer faces when making production decisions. Various methods can be used to reduce price variability and set a satisfactory price before products are ready for sale. Table 10 gives risk management strategies that farmers can apply against marketing and price risk. Accordingly, farmers evaluated the strategies of improving transportation and transportation facilities against marketing and price risk, having information about past crop prices, having information about the market where the crop will be sold, and improving storage facilities as very important. According to farmers in the farms examined, contract production is not important as a marketing risk management strategy.

Table 10. Marketing and Price Risk Management Strategies in the Examined Farms

Marketing and Price Risk Management Strategies	Mean	Std. Deviation	Scale (%) 1	2	3	4	5	Total
Improving transportation and transportation facilities	1.03	0.17	96.9	3.1	-	-	-	100.0
Having information about past crop prices	1.03	0.17	96.9	3.1	-	-	-	100.0
Having information about the market where the crop will be sold	1.28	0.52	75.0	21.9	3.1	-	-	100.0
Improving storage facilities	1.43	0.91	75.0	12.5	9.4	3.1	-	100.0
Spreading product sales over time	1.65	0.65	43.8	46.9	9.4	-	-	100.0
Becoming a partner in the cooperative	2.09	0.96	28.1	43.8	21.9	3.1	3.1	100.0
Sell the product by processing it	2.71	0.88	9.4	28.1	43.8	18.8	-	100.0
Making contract production (Marketing, production)	4.96	0.17	-	-	-	3.1	96.9	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

Sustainability of Agricultural Activity

Decisions regarding agricultural enterprises are made in line with the goals and objectives of the farmer. In order to make these decisions, farmers need to know the basic elements of decision making. Basic decision-making process or cycle; It consists of the stages of determining goals and objectives, determining methods to achieve goals and objectives, evaluating different opportunities and alternatives, selecting opportunities and alternatives, planning and implementing the selected opportunities and alternatives, reviewing and evaluating the selected opportunities (Kahan, 2008).

All of the producers within the scope of the research stated that they had agricultural insurance in the last five years and were registered in the farmer registration system. All of the farmers in the farms examined stated that they would not give up agricultural production under any circumstances. Farmers reported that 68.75% of the farms kept business records. People who are influential in decision-making regarding agricultural activities in the examined farms were investigated and the obtained data are given in Table 11. It has been determined that farmers are the dominant decision makers in decisions regarding investment, savings, financing and marketing in farms, the family decides together on non-farm matters (84.4%), and the farmer and the family decide together on decisions about expanding the business.

Table 11. People Influential in Decision Making Regarding Agricultural Activities in the Examined Farms (%)

Decision Type	Farmer	Farmer's Wife	Farmer's Children	Family Together	Total
Investment decisions	56.3	15.6	12.5	15.6	100.0
Savings decisions	59.4	12.5	12.5	15.6	100.0
Financing decisions	62.5	12.5	9.4	15.6	100.0
Decisions about the future	6.3	28.1	6.3	59.4	100.0
Business expansion decisions	43.8	6.3	6.3	43.8	100.0
Marketing decisions	84.4	-	6.3	9.4	100.0
Decisions regarding production pattern	-	-	-	100.0	100.0
Decisions regarding non-business matters	9.4	3.1	3.1	84.4	100.0

The reasons why farmers continued agricultural activities in the examined farms were also investigated, and the findings are given in Table 12. In this context, the surveyed farmers expressed the following as very important reasons for agricultural production: he has land for agricultural production, he can make a profit, it is difficult to do another job, he is his own boss, he likes to do agriculture, it is difficult to change jobs and there are no good opportunities outside of agriculture.

Table 12. Reasons of Farmers for Agricultural Production in the Farms Examined

Reasons	Mean	Std. Deviation	Scale (%) 1	2	3	4	5	Total
Having land for agricultural activity	1.00	0.00	100.0	-	-	-	-	100.0
Make a profit	1.00	0.00	100.0	-	-	-	-	100.0
It is difficult to do another job	1.00	0.00	100.0	-	-	-	-	100.0
Being your own boss	1.00	0.00	100.0	-	-	-	-	100.0
Enjoying doing agriculture	1.03	0.17	96.9	3.1	-	-	-	100.0
It is very difficult to change jobs	1.18	0.73	90.6	6.3	-	-	3.1	100.0
Lack of good opportunities outside agriculture	1.34	0.97	87.5	-	6.3	3.1	3.1	100.0
Helping the family with this work	2.31	1.40	43.8	15.6	12.5	21.9	6.3	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

The elements that are thought to help farmers continue agricultural activities in the farms within the scope of the research were determined using the Likert scale and the findings are given in Table 13. Among the factors that will help farmers continue their agricultural activities in the examined farms, the elements stated as very important are; finding a loan when needed, having government support, guaranteeing product prices, becoming widespread of contract production, agricultural engineers in agricultural organizations visiting the enterprise, providing support against natural disasters (flood, hail, frost, etc.), being informed about product prices in advance, infrastructure improving the facilities (road, communication, etc.), having/increasing storage services, not encountering difficulties in recruiting workers, increasing credit opportunities, agricultural engineers from pharmaceutical dealers visiting the business, having transportation services, not having too much debt, and having non-agricultural income.

Table 13. Elements that Can Help Farmers to Continue Agricultural Activities in the Examined Farms

Elements that Can Help Farmers to Continue Agricultural Activities	Mean	Std. Deviation	Scale (%)					
			1	2	3	4	5	Total
Finding a loan when needed	1.00	0.00	100.0	-	-	-	-	100.0
Having government support	1.00	0.00	100.0	-	-	-	-	100.0
Guaranteeing product prices	1.00	0.00	100.0	-	-	-	-	100.0
Becoming widespread of contract production	1.00	0.00	100.0	-	-	-	-	100.0
Agricultural engineers in agricultural organizations visiting the enterprise	1.03	0.17	96.9	3.1	-	-	-	100.0
Providing support against natural disasters (flood, hail, frost, etc.)	1.03	0.17	96.9	3.1	-	-	-	100.0
Being informed about product prices in advance	1.03	0.17	96.9	3.1	-	-	-	100.0
İnfrastructure improving the facilities (road, communication, etc.)	1.06	0.35	96.9	3.1	-	-	-	100.0
Having/increasing storage services	1.06	0.24	93.8	6.3	-	-	-	100.0
Not encountering difficulties in recruiting workers	1.09	0.39	93.8	3.1	3.1	-	-	100.0
İncreasing credit opportunities	1.09	0.53	96.9	-	-	3.1	-	100.0
Agricultural engineers from pharmaceutical dealers visiting the business	1.12	0.33	87.5	12.5	-	-	-	100.0
Having transportation services	1.15	0.57	90.6	6.3	-	3.1	-	100.0
Not having too much debt	1.18	0.78	93.8	-	3.1	-	3.1	100.0
Having non-agricultural income	1.50	0.84	65.6	25.0	3.1	6.3	-	100.0
Increasing the number of cooperatives for agricultural purposes	1.71	0.92	53.1	28.1	12.5	6.3	-	100.0
Training in marketing	1.93	0.84	37.5	31.3	31.3	-	-	100.0
Increasing/improving educational opportunities	2.03	1.03	34.4	40.6	15.6	6.3	3.1	100.0
Organizing agricultural training programs for young people	2.03	0.89	28.1	46.9	21.9	-	3.1	100.0
Making extension programs	2.18	1.28	37.5	34.4	6.3	15.6	6.3	100.0
Providing additional job opportunities for family members	2.65	1.20	25.0	12.5	40.6	15.6	6.3	100.0
Increasing non-agricultural job opportunities	2.71	1.05	12.5	31.3	31.3	21.9	3.1	100.0
Family members working outside the business	3.46	1.50	15.6	12.5	18.8	15.6	37.5	100.0
Having child care (home economics) services	3.84	1.54	12.5	15.6	3.1	12.5	56.3	100.0
Convenience in matters such as packaging supply	4.28	1.24	9.4	-	12.5	9.4	68.8	100.0
Information about packaging	4.56	0.91	3.1	-	9.4	12.5	75.0	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

In the research, the opinions of farmers in the surveyed farms regarding their future goals and objectives were evaluated (Table 14). In the research, farmers' future goals and objectives were examined using the Likert scale. Farmers expressed it as very important for the majority of the goals and objectives presented to farmers. Considering the importance of watermelon production for the research region, it can be said that the results obtained are not surprising and all elements are considered very important for the farmer. Some of the most important future goals and objectives of farmers in the farms examined are; to reduce risks, to be recognized among other farmers, to improve living standards, to increase income, to invest in the business, to expand the business, to obtain high quality products.

Table 14. Future Goals and Objectives of Farmers in the Examined Farms

Future Goals and Objectives of Farmers	Mean	Std. Deviation	Scale (%)					
			1	2	3	4	5	Total
To reduce risks	1.00	0.00	100.0	-	-	-	-	100.0
To be recognized among other farmers	1.00	0.00	100.0	-	-	-	-	100.0
To improve living standards	1.00	0.00	100.0	-	-	-	-	100.0
To increase income	1.00	0.00	100.0	-	-	-	-	100.0
To invest in the business	1.00	0.00	100.0	-	-	-	-	100.0
To expand the business	1.00	0.00	100.0	-	-	-	-	100.0
To obtain high quality products	1.00	0.00	100.0	-	-	-	-	100.0
Ability to produce contract production	1.00	0.00	100.0	-	-	-	-	100.0
Producing products in the quantity desired by the market	1.00	0.00	100.0	-	-	-	-	100.0
To be able to sell the product at the highest price in the market	1.00	0.00	100.0	-	-	-	-	100.0
Avoiding problems in marketing products	1.00	0.00	100.0	-	-	-	-	100.0
No problems in product transportation	1.00	0.00	100.0	-	-	-	-	100.0
Producing products of the quality desired by the market	1.03	0.17	96.9	3.1	-	-	-	100.0
Being open to innovations	1.06	0.24	93.8	6.3	-	-	-	100.0
Earn enough income	1.06	0.35	96.9	-	3.1	-	-	100.0
No problems with storage	1.09	0.29	90.6	9.4	-	-	-	100.0
Maintaining Living Standard	1.12	0.70	96.9	-	-	-	3.1	100.0
Protecting the environment while producing	1.15	0.72	93.8	3.1	3.1	-	-	100.0
Making time for activities outside of work	1.40	0.94	78.1	12.5	3.1	3.1	3.1	100.0
Reduce your workload	1.40	0.91	78.1	9.4	9.4	-	3.1	100.0
Ensuring that children continue farming in the future	1.43	1.14	81.3	9.4	-	3.1	6.3	100.0
Saving money for retirement	1.43	1.29	87.5	-	3.1	-	9.4	100.0
Becoming a partner in an agricultural cooperative	2.90	1.05	12.5	18.8	34.4	34.4	-	100.0
Saving money for children's education	3.18	1.97	37.5	6.3	6.3	-	50.0	100.0
No problems with packaging	4.59	0.83	3.1	-	3.1	21.9	71.9	100.0

Scale * *1:Very important, 2:Important, 3: Neutral, 4:Not important, 5:Not at all important*

Information Resources

Good risk management decisions are based on accurate information, which requires reliable data. Good information is one of the most useful elements a farmer can have to help him make rational risk management decisions. While there are many sources of information available, the most appropriate place to look for information depends on the type of risk the farmer needs to manage. It is possible to collect information sources that can be used in decision-making in agriculture under the headings of business records, non-business information sources and other information sources.

In case of any problems related to agricultural activities in the farms examined within the scope of the research, the people whose information can be used are stated as the headman, relatives, neighbors, other farmers, agricultural consultants, agricultural engineers of the provincial and district directorates of Agriculture and dealers. The frequency of meeting with experts on agricultural activities in the farms examined is once a week (59.4%) and once every fifteen days (40.6%) (Table 15).

Leaders in bringing innovations in agricultural activities in the farms within the scope of the research were examined and the findings are given in Table 16. In the farms examined, it is seen that 15.6% of the producers determine the cooperative, 6.3% the headman, and 56.3% the agriculturalist/extension personnel as the pioneer in bringing innovations. The issue of which communication tools are generally used regarding agriculture in the examined farms was investigated. Within the scope of the research, it was determined that 96.9% of the farmers benefited from the internet, all from television, 15.6% from newspapers and 9.4% from brochures (Chart 15).

Table 15. Information Sources in the Examined Farms

	Person	%
Frequency of Meeting with Experts on Issues Related to Agricultural Activities		
Once a week	19	59.4
Once in fifteen days	13	40.6
Total	**32**	**100.0**
Leading People in Bringing Innovations to Agricultural Activities *		
Farmer	6	18.8
Headman	2	6.3
Agricultural Engineer / Extension Staff	18	56.3
Cooperative	5	15.6
Provincial Directorate of Agriculture Personnel	7	21.9
Fertilizer, Seed Dealer	17	53.1
Communication Tools Used Regarding Agriculture *		
Television	32	100.0
Newspaper	5	15.6
Internet	31	96.9
Brochures	3	9.4

**More than one answer received.*

CONCLUSION

In agriculture, the ability of businesses to continue their activities successfully depends on preventing risks as much as possible and reducing their recurrence and impact. No matter how experienced those involved in agricultural production are in agriculture, they often face many risk factors. As mentioned before, there are many risk sources affecting agriculture. The main risk sources that often arise are diseases, natural conditions, fluctuations in the prices of products, etc. can be specified as. Knowing the risk sources encountered in agriculture and taking various precautions accordingly is an important issue.

In this study, socio-economic characteristics, risk sources, risk management strategies and sustainability of agricultural activities in watermelon producing farms in Karataş district of Adana province were investigated. All of the farmers in the farms examined stated that they would not give up agricultural production under any circumstances. The most important elements that will help farmers continue watermelon production in the farms within the scope of the research are; finding a loan when needed, having government support, guaranteeing product prices and increasing non-agricultural job opportunities. Primary goals and objectives of producers; to reduce risks, to sell the product at the highest price in the market, to have no problems in product transportation, and to produce products of the quality desired by the market.

In the study, risk sources that negatively affect watermelon production in the surveyed enterprises in Karataş district of Adana province;

- changes in product yields,
- low productivity due to plant diseases and pests,
- lack of information regarding the production method,
- inadequacy of agricultural tools and machinery,
- changes in climatic conditions,
- inadequacy of analysis laboratories,
- difficulties encountered in finding foreign labor,
- product losses occurring during harvest,
- changes in interest rates,
- changes in input costs,
- debt structure, indebtedness status,
- lack of financing,
- capacity and availability of credit resources,
- financing without equity capital,
- inflation,
- changes in watermelon prices,
- lack of knowledge about marketing and sales,
- lack of market guarantee for the products,
- problems encountered in storage and transportation,
- inability to grow products of suitable variety and quality for the market,
- health/disability/death status of the business owner or worker,
- changes in the economic situation of the country,
- lack or insufficient government support,
- determined as changes in the general and agricultural policies implemented by the government.

As a result, the findings obtained from the study reveal the problems and risks faced by watermelon producers. Risk management strategies that can be applied in the research area to mitigate the effects of these risks encountered in watermelon production and to ensure continuity in production and sustainability in businesses are;

- to produce at the lowest cost possible,
- getting crop insurance,
- fighting diseases with medicine,
- to diversify and differentiate the business,
- enlarging the business land,
- planning expenses,
- reduce debt,
- increase solvency,
- saving money, finding capital resources,
- making financial analysis,
- making investments outside the business,
- improving transport and transport facilities,

- having information about past product prices and the market,
- improving conservation opportunities.

REFERENCES

Adanacioglu, H., Olgun, A. 2010. Effects on the economic performance of framers of the risks encountered in the production of organic cotton, and risk management strategies: A Turkish case study. African Journal of Agricultural Research, 5 (24): 3387-3393.

Agir, H.B., Saner, G., Adanacioglu, H. 2015. Risk sources encountered by farmers in the open field production of strawberry and risk management strategies: A case of Menemen Emiralem District of Izmir. Journal of Agricultural Sciences, 21: 13-25.

Agir, H.B., Erdem, M. 2022. Perception of Wheat Farmers’ Risk and Risk Strategies: The case of Kahramanmaras Province. Turkish Journal of Agricultural and Natural Sciences, 9 (1): 203-210.

Ahmed, M.A., Haji, J., Legesse, B., Demisse, T. 2020. Risk perception and management in smallholder vegetable farming in Eastern Ethiopia. Journal of Natural Sciences Research, 10 (4): 1-12.

Akcaoz, H., Kizilay, H., Ozcatalbas, O. 2009. Risk management and sustainability in banana production: A case study from Turkey. Journal of Food Agriculture and Environment, 7 (2): 283-294.

Akcaoz, H.V., Suna R., San, M.M. 2024. Risk Management in Agricultural Enterprises in Çubuk District of Ankara Province. 4th International Congress of Engineering and Natural Sciences.

Akhtar, S., Gu-cheng, L., Ullah, R., Nazir, A., Iqbal, M.A., Raza, M.H., Iqbal, N., Faisal1, M. 2018. Factors influencing hybrid maize farmers’ risk attitudes and their perceptions in Punjab Province, Pakistan. Journal of Integrative Agriculture, 17(6): 1454–1462.

Akhtar, S., Gu-cheng, L., Nazir, A., Razzaq, A., Ullah, R., Faisal, M., Naseer, M.A.R., Raza, M.H., 2019. Maize production under risk: The simultaneous adoption of off-farm income diversification and agricultural credit to manage risk. Journal of Integrative Agriculture, 18(2): 460–470.

Ali, J., Kapoor, S. 2008. Farmers’ perception on risks in fruits and vegetables production: An empirical study of Uttar Pradesh. Agricultural Economics Research Review, 21: 317-326.

Almadani, M.I. 2014. Risk attitude, risk perceptions and risk management strategies: An empirical analysis of Syrian wheat-cotton and pistachio farmers. PhD Thesis. Faculty of Agricultural Sciences, Georg-August University, Gottingen, Germany.

Alp, C. 2017. Risk analysis in tomato farming agricultural businesses: Sample of Plain Kumkale in the City of Canakkale. Msc Thesis. Canakkale Onsekiz Mart University, Faculty of Science and Technology, Department of Agricultural Economics, Canakkale.

Bagheri, A., Fami, S. 2016. Potato growers’ risk perception: A case study in Ardabil Province of Iran. Journal of Agricultural Science and Technology, 18: 55-65.

Bal, D.C. 2018. The evaluation of risk sources and risk management strategies on crop production in trace region. Tekirdag Namik Kemal University Graduate School of Natural and Applied Sciences Department of Agricultural Economics, PhD Thesis, Tekirdag, Turkey.

Buyukkalay, H. 2019. Watermelon Production and Marketing Structure in Antalya Province. Akdeniz University Institute of Science, Department of Agricultural Economics, Master's Thesis, Antalya.

Cetin, I., Esengun, K. 2012.Measuring risk and designing risk management strategies for dry onion growing farms: A case study for Amasya Province of Turkey. Scientific Research and Essays, 7 (50): 4259-4268.

Gungor, M. 2006. Agricultural Insurance Practices and State Supported Agricultural Insurance in Turkey. Marmara University, Banking and Insurance Institute, Insurance Department, Master's Thesis, Istanbul.

Hakorimana, F., Akcaoz, H. 2020. Risk sources and risk management strategies in coffee farming: A case study of Rwanda. Turkish Journal of Agricultural Economics, 26 (1): 1-17.

Hayran, S. 2019. Perceptions of wheat producers towards risk and risk management strategies: A case study from Turkey. Ciencia Rural, 49 (12): 1-11.

Iqbal, M.A., Ping, Q., Abid, M., Muhammad, M.K.S., Rizwan, M. 2016. Assessing risk perceptions and attitude among cotton farmers: A case of Punjab Province, Pakistan. International Journal of Disaster Risk Reduction, 16: 68-74.

Iqbal, M.A., Ping, Q., Zafar, M.U., Abbas, A., Bashir, M.K., Ali, A., Kousar, R. 2018. Farm risk sources and their mitigation: A case of cotton growers in Punjab, Pakistan Journal of Agricultural Science, 55 (3): 677-684.

Kahan, D. 2008. Managing in Farming, FAO Farm Management Extension Guide, Rome.

Kaya, G. 2022. Watermelon Product Report. Agricultural Economics and Policy Development Institute / TEPGE.

Nicol, R.M., Ortmann, G.F., Ferrer, S.R.D. 2007. Perceptions of key business and financial risks by large-scale sugarcane farmers in KwaZulu-Natal in a dynamic socio-political environment. Agrekon, 46 (3): 351-370.

Ozer, D., Tumer, E.I. 2020. Analysis of the factor affecting the attitude of lemon producers against risk: The case of Erdemli District of Mersin Province. Turkish Journal of Agricultural and Natural Sciences, 7 (4): 988-996.

Pellegrino, J.M. 1999. Risk management in agriculture: Argentine evidence of perceived sources of risk, risk management strategies, and risk efficiency in rice farming. Master of Commerce (Agriculture) Thesis. Lincoln University.

Rizwan, M., Deyi, Z., Nazir, A., Osti, R., Ahmed, U.I., Zhu, Y., Iqbal, M.A. 2017. Risk perceptions and management strategies of rice growing households: Evidence from a Developing Country. Journal of Applied Environmental and Biological Sciences, 7 (3): 16-26.

Sookhtanlo, M., Sarani, V. 2011. Analysis of factor affecting on risk management of wheat production among wheat farmers (Razavich Region, Khorasan-E-Razavi Province, Iran). Agris online Papers in Economics and Informatics, 3 (4): 3-11.

TUIK, 2024. Turkish Statistical Institute, Data Porta for Statistics. https://data.tuik.gov.tr/Kategori/GetKategori?p=tarim-111&dil=1.

Ucar, K., Engindeniz, S. 2019. The risk sources of apricot production and applicable risk management strategies in Malatya Province. Igdir University Journal of the Institute of Science and Technology, 9 (4): 2313-2320.

Usman, J., Jirgi, A.J., Ojo, M.A., Tiamiyu, S.A. 2017. Sources of risk and management strategies among farmers in rice post-harvest management in Niger State, Nigeria. International Journal of Environmental and Agriculture Research, 3 (7): 60-66.

OPTIMISATION OF THE CHEMICAL COMPOSTING OF CATTLE MANURE BY MEANS OF NITRIC ACID

Rochdi Ghallab1, Amina Kemmouche2 and Brahim Kebabi3

1. National Polytechnic School of Constantine

2. National Higher School of Biotechnology of Constantine

3. Laboratory of Water Pollution and Treatment, Department of Chemistry, Faculty of Exact Sciences, Constantine 1-Frères Mentouri University

Corresponding author e-mail: brahim.kebabi@umc.edu.dz

ABSTRACT

The composting of cattle manure is a crucial process for transforming this organic waste into a soil amendment that is rich in nutrients. This practice plays an instrumental role in reducing the amount of waste sent to landfills and in mitigating greenhouse gas emissions. This environmentally friendly method sustainably enriches soils,which improves crop quality and plant health. There are two main types of composting: biological and chemical. While beneficial, biological composting is slow and requiresrigorous management of environmental conditions to optimise decomposition by microorganisms.In contrast, chemical composting is faster and more economical,where chemical additives are used to accelerate decomposition. In this study we investigated a chemical method using nitric acid to oxidise cattle manure.The excess acid was neutralised with potassium hydroxide, leaving harmless residues such aspotassium and nitrates, which are also powerful fertilisers. Scanningelectron microscopy and energy dispersive X-ray spectroscopy (SEM-EDX) analyses showed that the concentration of nitric acid had a significant effect on compost quality.The results show a decrease in carbon concentration and an increase in nitrogen and potassium concentrations with higher nitric acid concentrations used in the composting process, illustrating the oxidation of organic matter and the incorporation of nitrogen and potassium into thecompost. The C/N ratio, which is crucial for decomposition and plantnutrition, is optimal within a range of nitric acid concentrations from 0.05 Nto 1.00 N, facilitating the production of high-quality compost. The texture of thesamples analysed by SEM also varies with nitric acid concentration, with higher and more uniform degradation of organic matter and a finer texture with improved porosity.

Keyword: Cattle Manure Composting, Chemical Composting, SEM-EDX, Soil Amendments, Fertilizer Production.

INTRODUCTION

Cattle manure composting is a vital process for efficiently converting it into a highly beneficial, nutrient-rich soil amendment.(Larney et al., 2006). By reducing the amount of manure sent to landfills and greenhouse gas emissions, this practice promotes the recycling of organic materials and contributes to environmental preservation. The compost produced can be used to enrich soils in an ecological and economical manner, thereby improving crop quality and plant health.

Cattle manure composting can be achieved either biologically or chemically. Biological composting relies on the action of natural microorganisms, such as bacteria and fungi, which decompose the manure in the presence of oxygen. This aerobic process produces nutrient-rich compost that benefits soil structure and fertility. However, biological composting has some

drawbacks. It is much slower than chemical composting, requiring careful management of temperature, humidity, and aeration conditions to optimize the process. Additionally, due to handling and exposure to organic materials, there is a potential risk of pathogen spread, including viruses such as COVID-19, though this risk can be mitigated by proper composting practices and health safety measures (Zeqiri &Biçoku, 2021).
In contrast, chemical oxidation uses chemical additives to accelerate manure decomposition (Jensen et al., 2013). In this case, care must be taken to ensure that the chemicals used are not hazardous and that chemical residues in the compost do not affect its quality and environmental impact. Chemical composting is significantly more economical than biological composting.
In our study, aiming to replace the traditional composting process, we conducted the chemical oxidation of cattle manure using nitric acid. The excess nitric acid at the end of the process is neutralized using potassium hydroxide. The remaining residues of this process are potassium and nitrates, which are not only harmless but also constitute potent fertilizers, thereby enriching our compost.

MATERIALS AND METHODS

Scanning electron microscopy and elemental analyses

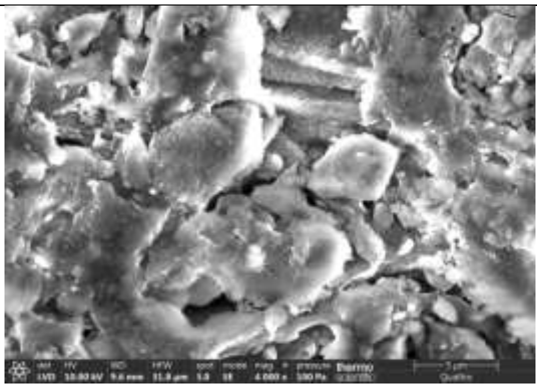
Scanning electron microscopy (SEM) images and elemental analyses were performed using a Thermo Fisher Scientific Helios G4 UC system, equipped with Energy Dispersive X-ray Spectroscopy (EDX). The EDX analysis was conducted at an accelerating voltage of 20 kV and a beam current of 1.6 nA, with a spectrum acquisition time of 60 seconds.

Compost Preparation

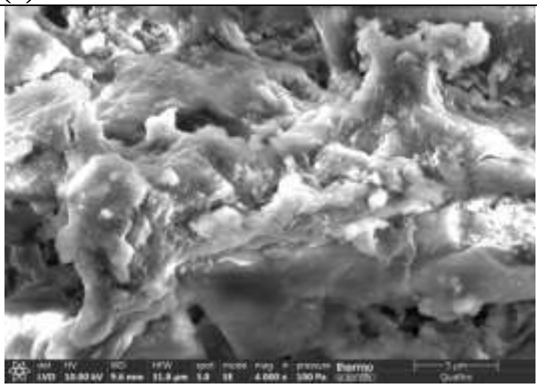
A fixed amount of collected manure is weighed before and after drying to determine the moisture content. Once this step is completed, the manure is ground using a ball mill to obtain a homogeneous texture. Particles smaller than 250 micrometers are recovered by sieving and then stored dry in bags for later use. Three grams of this dried and ground manure are mixed with 15 ml of nitric acid (at concentrations of 1 M, 0.5 M, 0.1 M, 0.05 M, and 14 M), until a paste is formed. Finally, the paste's pH is adjusted using a potassium hydroxide solution to achieve a neutral pH. The resulting product is dried for characterization.

RESULTS

The pH of the compost obtained for samples treated with nitric acid showed an acidic pH, ranging from 3 to 5 depending on the acid concentration used before neutralization with KOH. After neutralization, the pH was adjusted between 6.5 and 7.5. The texture of the obtained samples appears to depend on the concentration of nitric acid used during their preparation. Higher concentrations of nitric acid result in more uniform degradation of organic matter, leading to a finer texture with improved porosity.
The texture of the prepared samples is shown in Figure 1. Image (a) presents raw cattle manure at a magnification of x4000. Images (b), (c), (d), and (e), also at x4000 magnification, show cattle manure samples treated with nitric acid at concentrations of 0.05 M, 0.5 M, 1 M, and 14 M, respectively.

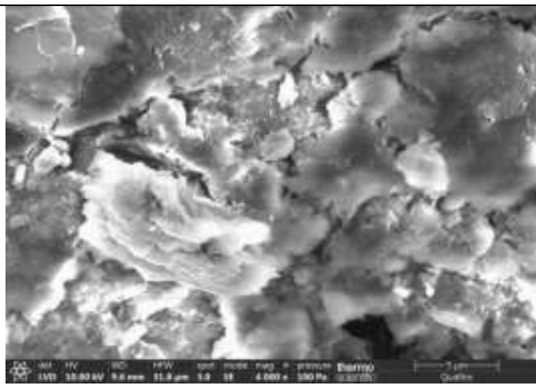


(a) Raw cattle manure



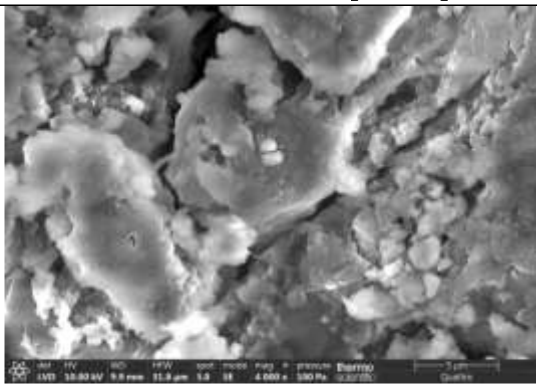
(b)

Cattle manure treated with $[HNO_3]$ = 0.05 M



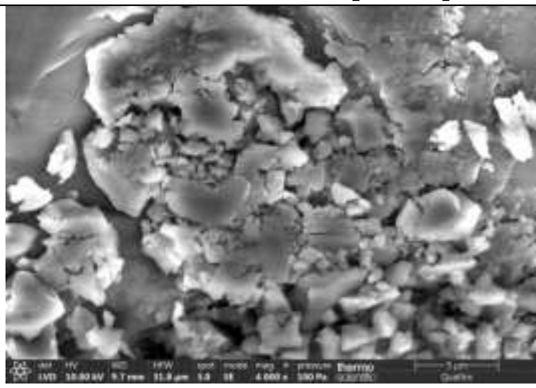
(c)

Cattle manure treated with $[HNO_3]$ = 0.5 M



(d)

Cattle manure treated with $[HNO_3]$ = 1 M



(e)

Cattle manure treated with $[HNO_3]$ = 14 M

Figure 1 SEM Images of (a) Raw cattle manure, (b) Cattle manure treated with [HNO3] = 0.05 M, (d) Cattle manure treated with [HNO3] = 1 M and (e) Cattle manure treated with [HNO3] = 14 M

SEM-EDX is often used for the characterization analysis of composts (Manohara et al., 2017, Diquattro et al., 2018,Khare et al., 2023). The images show the presence of two types of structures in each sample. The first consists of particles of varying sizes and shapes, which may be dispersed or clustered. The images also demonstrate the formation of particle aggregates, which are sets of interconnected particles, indicating the presence of organic matter.

The quality of the obtained product was characterized by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX). This technique was used to study the shape and structure of the compost samples as well as their chemical composition. The effect of nitric acid concentration on the quality of the obtained compost was the first parameter we

studied. For this purpose, we used the following concentrations: 0.05 M, 0.5 M, 1 M, and 14 M.
EDX analysis allowed us to identify the elements present in the compost, and the results are presented in Table 1. We focused particularly on the following elements: C, N, O, Al, Si, P, K, and Ca, which compose the compost. Special attention was given to C and N as they are key indicators of compost quality.

Table 1: EDX Analysis Results of raw cattle manure, cattle manure treated with $[HNO_3]$ = 0.05 M, cattle manure treated with $[HNO_3]$ = 0.5 M, cattle manure treated with $[HNO_3]$ = 1 Mand, cattle manure treated with $[HNO_3]$ = 14 M

Table 1: EDX analysis results of raw cattle manure and cattle manure treated with $[HNO_3]$ at concentrations of 0.05 M, 0.5 M, 1 M, and 14 M.

Element (%)	Raw cattle manure	$[HNO_3]$ = 0.05 M	$[HNO_3]$ = 0.5 M	$[HNO_3]$ = 1 M	$[HNO_3]$ = 14 M
C	40.21	43.87	36.68	38.30	8.51
N	0.08	4.97	6.47	5.63	10.07
O	42.67	41.89	39.92	45.94	39.16
Al	2.28	1.09	1.60	1.32	1.24
Si	5.42	1.05	1.65	0.96	0.18
P	0.77	1.37	2.24	0.78	0.12
K	1.01	1.22	7.62	2.04	39.67
Ca	7.57	3.67	2.31	3.04	0.39
C/N	502,63	8,83	5,67	6,80	0,85

The results show a decrease in carbon concentration with increasing nitric acid concentration during treatment, a consequence of the oxidation of organic matter. Nitrogen concentration in the compost increases in parallel with the nitric acid concentration used, which is expected as nitrogen comes from the acid. Additionally, potassium concentration in the compost increases with higher nitric acid concentrations because more potassium hydroxide is needed to neutralize the compost when the acid concentration is high. These observations highlight the significant impact of nitric acid concentration on the final chemical composition of the compost.
The C/N ratio decreases with the increase in nitric acid concentration used during oxidation. It remains favorable within a broad range of nitric acid concentrations from 0.05 N to 1.00 N.
Maintaining an optimal C/N ratio is essential for supporting decomposition and providing the necessary nutrients to plants after compost application (Azim et al., 2018). Achieving an optimal result over a wide range of nitric acid concentrations reduces manufacturing constraints.

CONCLUSION

This study highlights the effectiveness of chemical oxidation with nitric acid for cattle manure composting, demonstrating its potential to produce high-quality compost quickly and economically. Using nitric acid to oxidize manure, followed by neutralization with potassium hydroxide, not only accelerates the decomposition of organic matter but also enriches the compost with essential nutrients such as nitrogen and potassium. SEM and EDX analyses revealed that nitric acid concentration plays a crucial role in the final compost quality, influencing both its chemical composition and texture. In particular, the C/N ratio, a key indicator of compost quality, was found to be optimal within a range of nitric acid concentrations from 0.05 N to 1.00 N. This flexibility in nitric acid concentrations facilitates the production of high-quality compost without requiring extremely precise control of composting conditions. Chemical oxidation with nitric acid represents a promising method for valorizing cattle manure, providing a practical and eco-friendly solution for agricultural waste management and soil improvement.

REFERENCES

Azim, K., Soudi, B., Boukhari, S., Perissol, C., Roussos, S., & Thami Alami, I. (2018). Composting parameters and compost quality: a literature review. *Organic agriculture, 8*, 141-158.

Diquattro, S., Garau, G., Lauro, G. P., Silvetti, M., Deiana, S., & Castaldi, P. (2018). Municipal solid waste compost as a novel sorbent for antimony (V): adsorption and release trials at acidic pH. *Environmental Science and Pollution Research*, *25*, 5603-5615.

Jensen, L. S., & Sommer, S. G. (2013). Manure organic matter–characteristics and microbial transformations. *Animal manure recycling: Treatment and management*, 67-90.

Khare, S., Singhal, A., Mishra, A., & Das, B. (2023, January). An Assessment of Qualitative and Quantitative Municipal Solid Waste City Compost by Indexing Method. In *Invitation to National Level Conference on Sustainable Waste Management Practices* (pp. 47-64). Singapore: Springer Nature Singapore.

Larney, F. J., Sullivan, D. M., Buckley, K. E., &Eghball, B. (2006). The role of composting in recycling manure nutrients. *Canadian Journal of Soil Science*, *86*(4), 597-611.

Manohara, B., &Belagali, S. L. (2017). Evaluation of energy dispersive scanning electron microscopy and X-ray fluorescence techniques for analysis of compost quality. *Analytical Methods*, *9*(2), 253-258.

Zeqiri, M., &Biçoku, Y. (2021). Waste management in dairy farms during the COVID-19 period-the case of Gjilan region-Kosovo. *Scientific Papers. Series D. Animal Science, 64*(1).

SEASONAL DISTRIBUTION OF PROTIST PATHOGENS IN *Plodia interpunctella* POPULATIONS IN TÜRKİYE

Tuğba Sağlam Güvendik[1,2], *Mustafa Yaman*[1], *Ömer Ertürk*[2]

[1]*Department of Biology, Faculty of Arts and Science, Bolu Abant İzzet Baysal University, 14030, Bolu, Türkiye*
[2]*Department of Molecular Biology and Genetics, Faculty of Arts and Science, Ordu University, Türkiye*
Corresponding author e-mail: tugbasaglam32@hotmail.com

ABSTRACT

The Indian meal moth (*Plodia interpunctella*, (Lepidoptera; Pyralidae)) is one of the most economically significant pests of stored products globally. Stored product pests cause direct or indirect damage to contaminated food products. This pest lead to weight losses in products, deterioration of seed qualities, and degradation of quality and nutritional values, thereby negatively affecting the national economy. Interest in using entomopathogen as an alternative to chemical insecticides for the control of the pest is increasing day by day. Among entomopathogens, protist pathogens play a significant role as natural suppressive factors in pest insect populations. This study aimed to determine the seasonal distributions of protist pathogens in different populations of *P. interpunctella* in Türkiye during the five years (from 2019 to 2023). During the study, 6.367 *P. interpunctella* larvae, pupae, and adults (4.091 dead larvae, 609 living larvae, 1.330 adults, and 337 pupae) were dissected and examined under a light microscope from 14 different provinces in Türkiye (Ankara, Aydın, Bolu, Denizli, Gaziantep, Isparta, Istanbul, Izmir, Kastamonu, Malatya, Ordu, Samsun, Siirt, and Trabzon). As a result. microsporidian, neogregarine, and coccidian pathogens were observed during the dissections. When the seasonal distributions of microsporidia and neogregarine pathogens were examined seasonally, and significant differences were detected in each season of the year. However, coccidian pathogens could not be analyzed seasonally as they were not observed in different periods. This study is the first to examine the seasonal distribution of protist pathogens in *P. interpunctella* populations in Türkiye in detail. The results confirmed once again that *P. interpunctella* populations in Türkiye contain a considerable amount of protist entomopathogens.

Keywords: *Plodia interpunctella*, protist pathogen, seasonal distribution, Türkiye.

INTRODUCTION

The Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), holds a significant position among pests that cause substantial economic damage to stored products (Rees, 2004). *P. interpunctella*, which infests products and leads to both damage and degradation of nutritional quality, feeds and causes damage only during its larval stage. During feeding, it produces silk webs, contaminating food materials. As a result, the infested products suffer from weight loss, reduced commercial value, and decreased seed quality (TAGEM, 2008; Kaymakçı, 2022). The majority of the products damaged by *P. interpunctella* are foods consumed by humans. Fumigation with chemical insecticides is not considered suitable due to the harmful side effects on the environment and human health. Additionally, *P. interpunctella* has been reported to show resistance to many insecticides (Attia, 1977). Due to these impacts, it has become necessary to develop eco-friendly control methods that have no harmful side effects on the environment and non-target organisms. The most promising results in controlling *P. interpunctella* have been obtained from trials using its natural enemies (Schöller and Filinn, 2000; Grieshop, 2005; Shojaaddini et al., 2012). Interest in the use of entomopathogens as an alternative to chemical insecticides in controlling this pest is increasing day by day (Yaman, 2012). Among entomopathogens, protist pathogens play a significant role as natural suppressive factors in pest insect populations. This study aims to determine the seasonal distributions of protist pathogens in different populations of *Plodia interpunctella* in Turkey over a period of five years (2019-2023).

MATERIAL AND METHOD

During the study, 6.367 specimens including larvae, pupae and adults of *P. interpunctella* were collected from fourteen regions (Ankara, Aydin, Bolu, Denizli, Gaziantep, Isparta, İstanbul, İzmir, Kastamonu, Malatya, Ordu, Samsun, Siirt, Trabzon) of Türkiye (Table 1).

Table 1. Sampling localities and dates for *Plodia interpunctella* populations

Locality	Sampling Date
Ankara	08.07.2021, 18.02.2022, 05.04.2023
Aydın	12.06.2019, 02.07.2019, 22.07.2019, 18.06.2020, 30.06.2020, 22.06.2021, 12.07.2021, 14.05.2022
Bolu	22.05.2019, 28.06.2019, 08.07.2019, 20.08.2019, 05.09.2019, 05.09.2019, 12.09.2019, 20.01.2020, 18.02.2020, 11.03.2020, 23.03.2020, 30.04.2020, 01.06.2020, 13.07.2020, 17.06.2021, 12.07.2021, 24.03.2022, 31.05.2022, 15.06.2022; 20.01.2023; 05.09.2023; 12.05.2023
Denizli	01.06.2019, 28.06.2019
Gaziantep	05.07.2019, 05.08.2019, 11.09.2019, 22.07.2020, 27.07.2020, 25.07.2021; 13.07.2023
Isparta	02.05.2019, 13.07.2019, 06.08.2020, 31.08.2020, 26.05.2021, 09.05.2022, 08.06.2023
İstanbul	20.12.2019, 16.03.2020, 02.04.2020, 08.02.2023
İzmir	12.06.2019
Kastamonu	28.07.2021
Malatya	13.06.2019, 21.06.2019, 12.09.2019, 16.07.2020, 20.08.2020, 18.07.2021, 13.07.2023
Ordu	18.06.2019, 21.06.2020
Samsun	10.06.2019, 10.07.2020, 28.07.2021, 25.07.2023
Siirt	28.06.2019
Trabzon	15.06.2019, 10.07.2020, 28.07.2021, 25.07.2023

Microscopic Examination

P. interpunctella larva, pupa and adult samples were dissected in Ringer's solution and then prepared wet smears including host fat body, malpighian tubules, gut and hemolymph were examined for presence of protist infections under a light microscope at a magnification of 400–1000 ×. When an infection was found, the slides were air-dried and fixed with methanol, then stained with freshly prepared 5% solution of Giemsa stain. They were then washed in running tap water, air-dried and examined under a microscope.

RESULTS AND DISCUSSION

During the study, 6.367 of *P. interpunctella* samples including larvae, pupae and adults were dissected and searched for protist pathogens such as microsporidia, coccidia and neogregarines in the fourteen localities of Turkey between the years 2019-2023. Protist infections were confirmed by observation of their characteristic spores/oocysts. Three different protist pathogens, microsporidium, coccidian and neogregarine were observed in *P. interpunctella* populations.

The seasonal density of the microsporidia pathogen was examined in detail. According to the data obtained throughout the study, the infection rate was quite high in the first six months of 2019 (January, February, March, April, May, and June), reaching 42%, but it was found to drop to as low as 20% in the second half of the year (Figure 1).

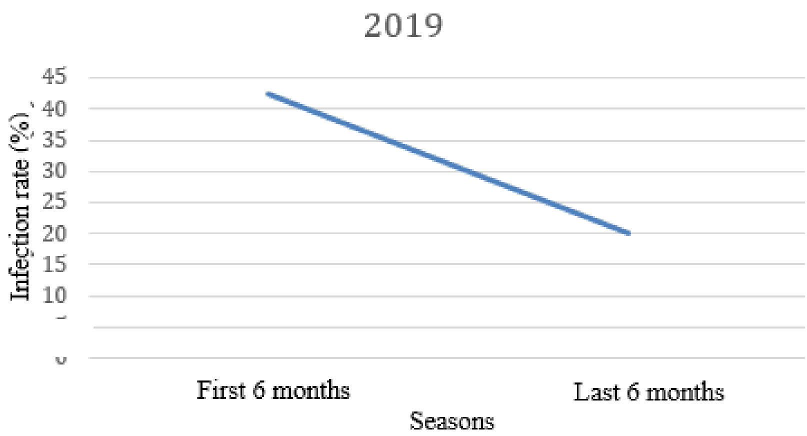


Figure 1. The microsporidia density detected in 2019

In the first six months of 2020, the infection rate was observed to be 13%, while in the last six months of the year, it increased to as much as 29% (Figure 2).

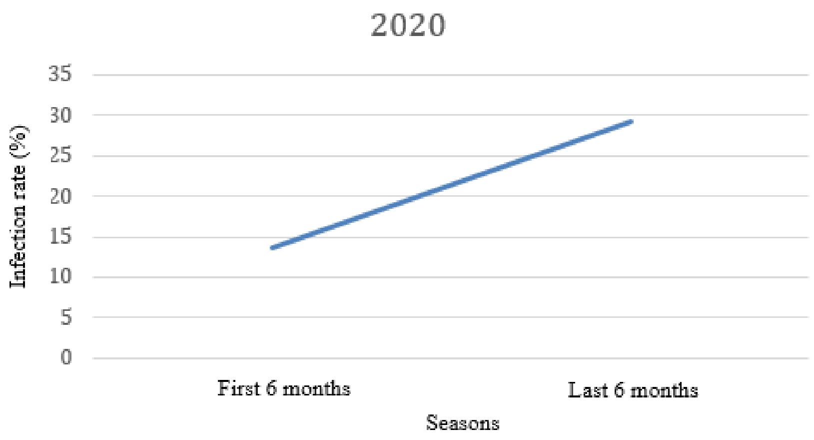


Figure 2. The microsporidia density detected in 2020

When looking at the infection intensity in 2021, it was observed that, similar to 2019, the infection rate was high in the first six months of the year, followed by a decline in the last six months (Figure 3).

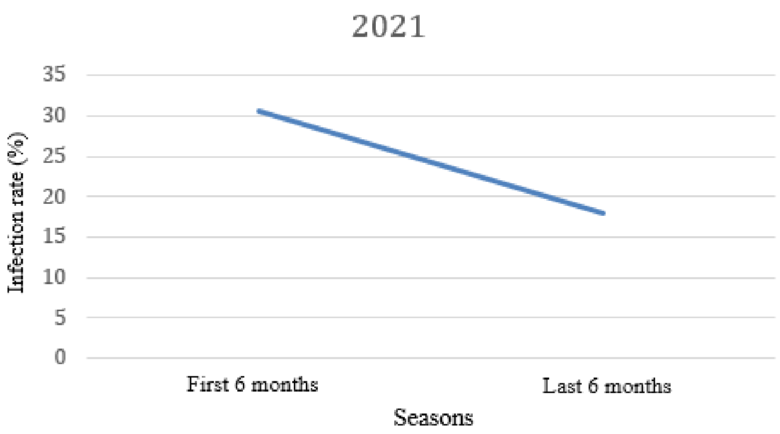


Figure 3. The microsporidia density detected in 2021

When examining the infection rate in 2022, it was found that, similar to 2019 and 2021, the infection was high in the first half of the year, followed by a slight decrease in the second half (Figure 4).

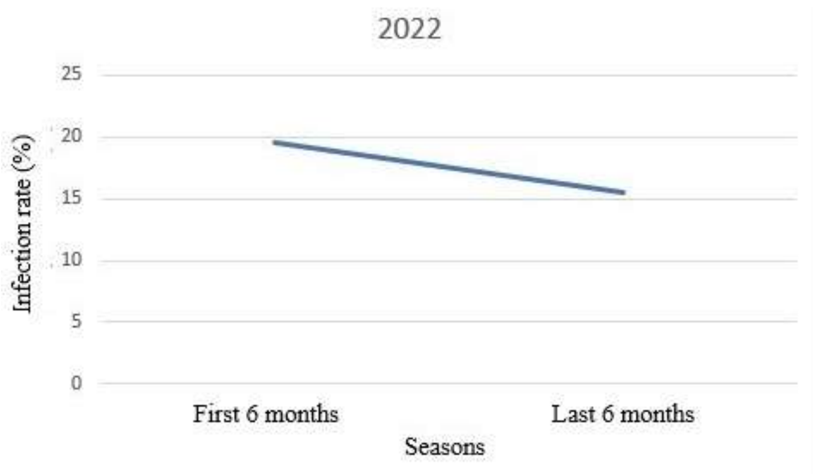


Figure 4. The microsporidia density detected in 2022

In 2023, the infection rate was found to be consistent, with the same level of infection observed in both the first and second halves of the year (Figure 5).

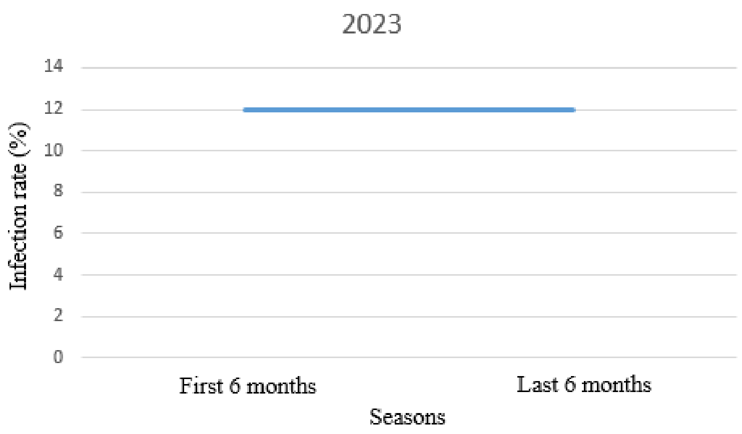


Figure 5. The microsporidia density detected in 2022

It is known that the development of *P. interpunctella* is influenced by temperature, with development accelerating at optimal temperature levels and slowing down at temperatures below this level. In storage facilities or homes where temperature is uncontrolled, *P. interpunctella* larvae are known to enter a diapause stage during cold months. However, when favorable environmental conditions are restored (especially in early spring or when homes remain warm even during winter months), a significant increase in moth populations occurs (Mason, 2002; Güngör, 2014). It has also been reported that if the temperature does not drop too low, the moth does not enter the diapause stage (Prevett, 1971). In our study, seasonal findings of *V. plodiae* isolate TR density generally showed a high infection density in the first six months of the year (Figures 1, 3, 4, and 5). This increase is thought to be due to the temperature and humidity levels in home and storage environments. When looking at the infection density in the last six months of the year, an increase in the infection rate was observed in 2020 (Figure 2). It is believed that the increase in infection during these months was due to the provision of optimal temperature conditions for *P. interpunctella* development, given that these are the warm months of the year. Throughout the study, it was possible to find heavily infested products even in winter, especially in field studies conducted in homes.

When the seasonal density of the neogregarine pathogen was examined, it was found that infection density increased in both the first and second halves of the year in 2019, 2020, 2021, and 2023 (Figures 6, 7, 8, and 10). This continued high infection rate in the second half of the year is also thought to be due to the optimal temperature conditions in home environments, which accelerate the development of *P. interpunctella*. Only in 2022 was it observed that the infection density decreased from 19% in the first half of the year to 15% in the second half (Figure 9).

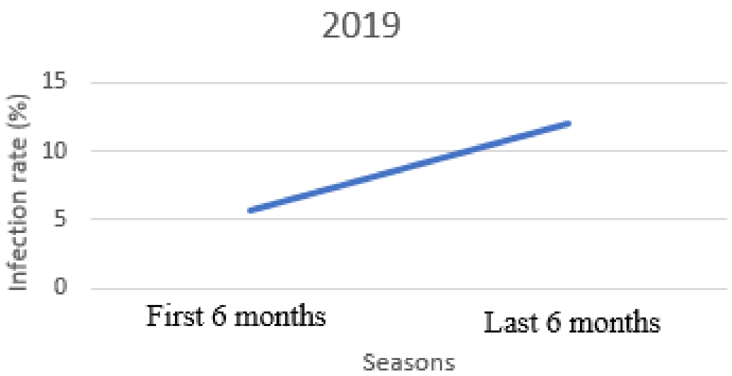


Figure 6. The neogregarine density detected in 2019

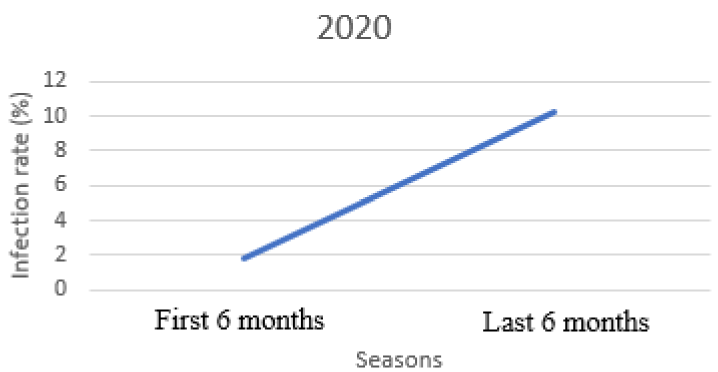


Figure 7. The neogregarine density detected in 2020

Figure 8. The neogregarine density detected in 2021

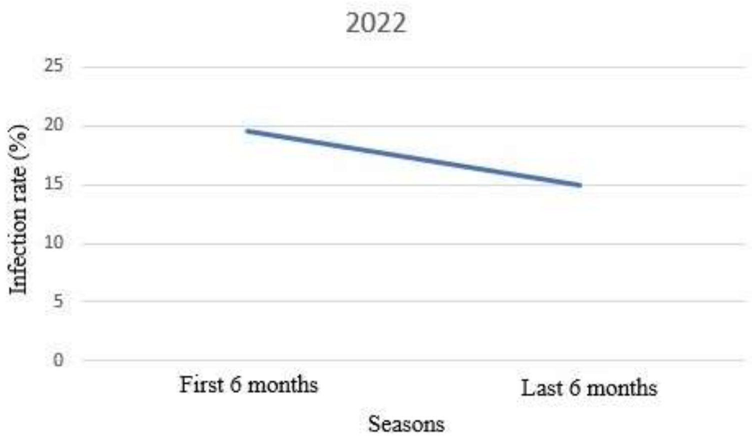


Figure 9. The neogregarine density detected in 2022

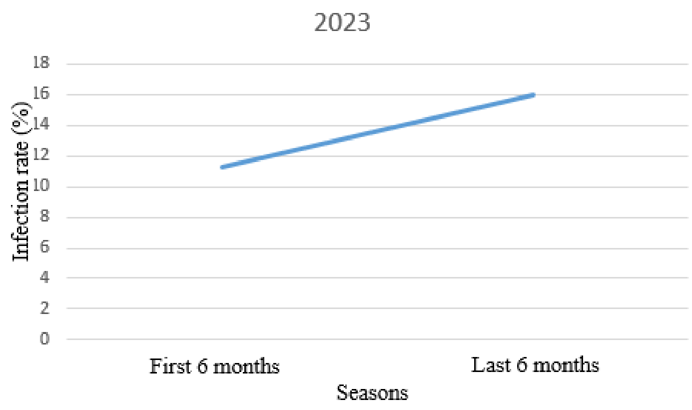


Figure 10. The neogregarine density detected in 2023

According to the study results, seasonal variations in the density of the detected pathogens were observed. These changes could be related to population density, and it is speculated that in dense populations, the rate of infection might be higher due to the increased horizontal transmission within the population.

When the seasonal distributions of the microsporidia and neogregarine pathogens were examined (the first six months of the year: January, February, March, April, May, June and the last six months of the year: July, August, September, October, November, December), significant differences were observed in each season. However, the coccidia pathogen could not be analyzed seasonally as it was not detected during different periods, making analysis impossible.

CONCLUSIONS

This study is the first to detail the seasonal distribution of protist pathogens in *P. interpunctella* populations in Turkey. The results confirm once again that *P. interpunctella* populations in our country contain a significant density of protist entomopathogens. McLaughlin (1971) noted that protozoan pathogens are generally the most suitable organisms for long-term pest population control. The current study has identified that the entomopathogenic protists detected are significant suppressive factors in the natural populations of the pest and are found to be widespread throughout the country. The obtained results encourage the use of protists in biological control against *P. interpunctella*.

ACKNOWLEDGMENT

This study was supported by Scientific and Technological Research Council of Türkiye (TUBITAK) under the Grant Number 118O980. The authors thank to TUBITAK for their supports.

REFERENCES

Attia, F.I. 1977. Insecticide resistance in *Plodia interpunctella* (Lepidoptera: Pyralidae) in New South Wales, Australia. *Aust. J. Entomol.* 16: 149–152.

Grieshop, M.J. 2005. Evaluation of three species of *Trichogramma* egg parasitoids for biological control of the Indian meal moth in warehouses and retail stores. Ph.D. Thesis, Kansas State University.

Güngör, F.B. 2014. Kuru Meyve Güvesi (*Plodia interpunctella* (Hübner), Lepidoptera: Pyralidae)'nde patojenik bir mikrosporidyum türünün karakterizasyonu. Yüksek Lisans Tezi, Trabzon Karadeniz Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı.

Kaymakçı, A.B. 2022. *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)'nın tam buğday unu, kepekli un ve buğday rüşeyminde gelişimi ve çoğalması. Yüksek Lisans Tezi, Konya Selçuk Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı.

Mason, L. 2002. Insects and mites: Food plant sanitation, Boca Raton, Florida, ABD.

McLaughlin, R.E. 1971. Use of protozoans for microbial control of insects. Burges, HD Microbial control of insects & mites.

Prevett, P.F. 1971. Some laboratory observations on the development of two African strains of *Plodia interpunctella* (Hübn.) (Lepidoptera: Phycitidae), with particular reference to the incidence of diapause. *J. Stored Prod. Res.* 7(4):253-260.

Rees, D. 2004. Insects of Stored Products. Collingwood: CSIRO Publishing.

Schöller, M., Flinn, P.W. 2000. Parasitoids and predators: Alternatives to Pesticides in Stored-product IPM, Ed.: Subramanyam, B., Hagstrum, DW., Kluwer Academic Publishers, Norwell, MA.

Shojaaddini, M., Lopez, M.J., Moharramipour, S., Khodabandeh, M., Talebi, A.A., Vilanova, C., Latorre, A., Porcar, M. 2012. A *Bacillus thuringiensis* strain producing epizootics on *Plodia interpunctella*: A case study. *J. Stored Prod. Res.* 48: 52-60.

TAGEM. 2008. Zirai Mücadele Teknik Talimatları. Başak Matbaacılık ve Tan. Hiz. Ltd. Şti., Türkiye, Ankara, 283.

Yaman, M. 2012. Böcek patolojisi atlası (Biyolojik mücadelede entomopatojenler için), Sage, Türkiye, Ankara.

THE PRESENCE OF PROTISTAN PATHOGENS IN SOME CHRYSOMELIDAE PESTS IN GEORGIA WITH THE COMPARISON THEIR OCCURRENCE IN TÜRKİYE

Mustafa Yaman[1], Manana Kereselidze [2,3], Tuğba Sağlam Güvendik[1]

[1]Department of Biology, Faculty of Arts and Science, Bolu Abant İzzet Baysal University, 14030, Bolu, Türkiye
[2]Scientific Research Center of Agriculture, Tbilisi, Georgia
[3]Vasil Gulisashvili Forest Institute, Agricultural University of Georgia, Tbilisi, Georgia
Corresponding author e-mail: tugbasaglam@gmail.com

ABSTRACT

Different geographic and climatic conditions can create very favorable environments for the development of many plant pests. The protection of agricultural products is very important both to ensure food safety and to contribute to the country economy. The interest in the use of entomopathogens (EPOs) as an alternative to chemical insecticides in the control strategies against plant pests is increasing day by day. Among the entomopathogens, protistan pathogens play an important role as natural suppressor factor in pest insect populations. The present study includes presence of protistan pathogens in the populations of three important Chrysomelidae pests, *Chaeotocnema tibialis*, *Phyllotreta atra* and *Leptinotarsa decemlineata* from the village Tsilkani in Mtskheta, Georgia. During the study, 160 samples of Chrysomelidae pests were dissected and searched for protistan entomopathogens. Gregarine pathogens were found in the populations of two Chrysomlidae pests, *Chaeotocnema tibialis* and *Phylloteta atra* in Georgia for the first time. However, any infection was not found in the examined *Leptinotarsa decemlineata* population. The gregarine infection rates were considerably low, 14% for *P. atra* and 15.6% for *C. tibialis* when compared with their occurrence in the populations of the both pests, 8.6-70.3% in *P. atra* and 63.2% in *C. tibialis*, in Türkiye. The present study includes the first records on the occurrence and prevalence of gregarine pathogens in the populations of Chrysomelidae pests in the village Tsilkani in Mtskheta Georgia. However it is needed to increase the number of populations and samples from different locations to represent the entire Georgia. Furthermore, it is needed to investigate other pathogens and parasites and identify each of them at the species level.

Keywords: Chrysomelidae pests, *Chaeotocnema tibialis*, *Phylloteta atra, Leptinotarsa decemlineata*, Georgia

INTRODUCTION

Chrysomelidae is one of the most species-rich and species-diverse groups of the order Coleoptera. This family is commonly known as leaf beetles and has approximately 36000 species in more than 2000 genera into 12 subfamilies (Bouchard et al. 2017). Leaf beetles are insects that depend on the area and structural features of the habitat. Therefore, increasing plant diversity is thought to increase the richness of these insects (Teles et al., 2020). Leaf beetles (family Chrysomelidae) primarily feed on the leaves of plants and they can cause significant damage quickly when occurring in large numbers. This may become an undesirable situation in agricultural and forest areas and Chrysomelidae species may become widespread agricultural pests. On the other hand, different geographic and climatic conditions can create very favorable environments for the development of many plant pests. Like other insect groups, leaf beetles are affected differently by different habitats.

Today, agricultural pests are generally controled with chemical pesticides. Chemical pesticides cause insecticide resistance, environmental pollution, toxicity to mammals and other non-target animals (Isman, 2006). Common use of chemical insecticides result in increasing attention given to natural products. Alternative control methods are needed for an effective and efficient fight against plant pests (Freitas et al., 2020).

Biological control using entomopathogens is a promising alternative control. Among the entomopathogens, protistan pathogens can play an important role as natural suppressor factor in pest insect populations in natural conditions. Therefore, studying the presence of protistan entomopathogens in pest populations can provide useful information for detecting natural factors in pest populations.

The present study includes presence of protistan pathogens in the populations of three important Chrysomelidae pests, *Chaeotocnema tibialis*, *Phyllotreta atra* and *Leptinotarsa decemlineata* in Georgia.

MATERIAL AND METHOD

160 adult samples of three important Chrysomelidae pests, *Chaeotocnema tibialis*, *Phyllotreta atra* and *Leptinotarsa decemlineata* were collected from the village Tsilkani in Mtskheta in Georgia. After macroscopic examination, they were dissected in Ringer's solution and wet smears were prepared. Host fat body, malpighian tubules, gut epithelium, and hemolymph were examined for the presence of the protistan entomopathogens under a light microscope at × 400–1000 magnification (Yaman et al., 2019). When an infection with the spore/oocysts of pathogen was observed, a part of the material was used for the preparation of Giemsa-stained smears. Giemsa-stained slides were examined under a light microscope.

RESULTS AND DISCUSSION

The members of the family Cyrysomelidae are frequently infected by protistan entomopathogens. Protistan entomopathogens infecting Cyrysomelidae have aroused interest as potentially suppressing factors of chrysomelid populations. For that, several new protistan entomopathogen species have been isolated and characterized from these insects recently. Geographic conditions and host plants may influence the diversity of protistan entomopathogens in insects. Therefore, the protistan entomopathogens of three important Chrysomelidae pests, *Chaeotocnema tibialis*, *Phyllotreta atra* and *Leptinotarsa decemlineata* in Georgia were determined and compared with the presence of their infections in Türkiye.

During the study, 160 samples of three important Chrysomelidae pests were dissected and searched for protistan entomopathogens. Gregarine pathogens were found in the populations of two Chrysomelidae pests, *C. tibialis* and *P. atra* in the village Tsilkani, Georgia for the first time. Seven of 45 *C. tibialis* beetles examined (15.6) in the population of *C. tibialis* collected in Tsilkani , Georgia were found to be infected by the gregarine pathogen. The gregarine infection was considerably low (15.6%) for *C. tibialis* populations in Georgia when compared with its occurrence in *C. tibialis* populations in Türkiye (Table 1). Yaman (2004) observed gregarine infection in 96 of 152 beetles examined (63.2%) in the population of *C. tibialis* in Trabzon, Türkiye.

The gregarine infection was also considerably low (14%) for *P. atra* population when compared in Georgia when compared with its occurrence in *P. atra* populations in Türkiye (Table 1).

Yaman (2004) observed considerable high gregarine infection reaching 70.3% in the populations of *P. atra* at different localities of the Middle and East Black Sea Region of Türkiye.
On the other hand, no protistan entomopathogen other than the gregarine pathogen were observed in the examined *P. atra* and *C. thibialis* beetles in Georgia.. Contrast, two new microsporidian species, *Nosema chaetocnema* (Yaman and Radek, 2003) and *N. tokati* (Yaman et al., 2008) were recorded from *C tibialis* populations in Türkiye. Furthermore, already known microsporidium species, *Nosema phyllotretae* was recorded from *P. atra* populations in Türkiye (Yaman et al., 2005).

During the study, we also investigated protistan entomopathogens *Leptinotarsa decemlineata* population inGeorgia. For this, 15 larvae and 50 adults of *L. decemlineata* were examined for any protistan entomopathogens. Unfortunately, any infection was not found in the examined larvae and adults of *L. decemlineata* (Table 1). However, Yaman et al. (2011) recorded the microsporidium pathogen, *Nosema leptinotarsae* in *L. decemlineata* population in Türkiye. Yaman et al. (2011) found the microsporidian pathogen in both larvae and adults of *L. decemlineata*.

Table 1. Protistan entomopatogen infection in three important Chrysomelidae pests, *Chaeotocnema tibialis*, *Phyllotreta atra* and *Leptinotarsa decemlineata* from village Tsilkaniin Mtskheta, Georgia.

Pest species	Number of examined samples	Number of infected samples	Infection rate (%)	Infection rates in Türkiye
Chaeotocnema tibialis	45[a]	7[g]	15.6	63.2[g] (Yaman, 2004)
Phyllotreta atra	50[a]	7[g]	14	8.6-70.3[g] (Yaman, 2002)
Leptinotarsa decemlineata	50[a]	0	0	1.89[n] (Yaman et al. 2011)
	15[l]	0	0	4.51[n] (Yaman et al. 2011)

[g] Gregarine infection, [n] Nosema, [a] Adult, [l] larva

CONCLUSIONS

The present study includes the first records on the occurrence and prevalence of gregarine pathogens in the populations of Chrysomelidae pests in Georgia. When compared the infection rates and infective pathogens, it is seen that it is a considerable difference in the term of infection rates and infective protistan pathogen species between the populations of three important Chrysomelidae pests, *Chaeotocnema tibialis, Phyllotreta atra* and *Leptinotarsa decemlineata* in Georgia and Türkiye. However, it is needed to increase the number of populations and samples from different locations to represent the entire Georgia. Furthermore, it is needed to investigate other pathogens and parasites and identify each of them at the species level.

REFERENCES

Bouchard, P., Smith, A.B.T., Douglas, H., Gimmel, M.L., Brunke, A.J., Kanda, K. 2017. Biodiversity of Coleoptera. In: Insect Biodiversity: Science and Society. Editors: R.G Foottit, P.H. Adler, New York, John Wiley & Sons, 337-162.

Freitas, A.C.O., Gigliolli, A.A.S., Caleffe, R.T.C., Conte, H. 2020. Insecticidal effect of diatomaceous earth and dolomite powder against Corn weevil *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae). *Turk. J. Zool.* 44: 490-497.

Isman, M. 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annu. Rev. Entomol*. 51: 45–66.

Teles, T.S., Valente-Neto, F., Ribeiro, D.B., Raizer, J., Linzmeier, A.M. 2020. High turnover of Chrysomelidae (Coleoptera) species in semideciduous forest remnants in an agricultural landscape. *An. Acad. Bras. Ciênc.* 92(2):1. p.e20190745-e20190745.

Yaman, M. 2002. *Gregarina phyllotretae* Hoshide 1953, a protozoan parasite of the flea beetles, *Phyllotreta undulata* and *P. atra* (Coleoptera: Chrysomelidae) in Turkey, *Appl. Entomol. Zoolog.* 37:649-653.

Yaman, M., Radek, R. 2003. *Nosema chaetocnemae* sp. n. (Microspora; Nosematidae), a microsporidian parasite of *Chaetocnema tibialis* (Coleoptera: Chrysomelidae). *Acta Protozool.* 42: 231-237.

Yaman, M. 2004. A newly recorded gregarine parasite of *Chaetocnema tibialis* (Coleoptera, Chrysomelidae) from Turkey. *Turk.J. Zool.* 28:95-96.

Yaman, M., Radek, R., Linde, A., Özcan, N., Lipa, J.J. 2011. Ultrastructure, characteristic features and occurrence of *Nosema leptinotarsae* Lipa 1968, a microsporidian pathogen of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). *Acta Parasitol.* 56:1-7.

Yaman, M., Radek, R., Aslan, I., Ertürk, Ö. 2005. Characteristic features of *Nosema phyllotretae* Weiser 1961, a microsporidian parasite of *Phyllotreta atra* (Coleoptera: Chrysomelidae) in Turkey. *Zool. Stud.* 44:368-372.

Yaman, M., Radek, R., Toguebaye, B. 2008. A new microsporidian of the genus Nosema, parasite of *Chaetocnema tibialis* (Coleoptera: Chrysomelidae) from Turkey. *Acta Protozool.* 47:279-285.

Yaman, M., Algı, G., Radek, R. 2019. Morphological, ultrastructural and molecular identification of a new microsporidian pathogen isolated from *Crepidodera aurata* (Coleoptera, Chrysomelidae). Turk. J. Zool. 43: 407-415.

ROLES OF BENTHIC MACROINVERTEBRATES IN THE FOOD WEB

Nurcan Ozkan[1]

[1] *Trakya University, Education Faculty, Mathematics and Sciences Education Department, Edirne, Turkey*
Corresponding author e-mail: nurcanozkan@hotmail.com

ABSTRACT
Benthic macroinvertebrates are small animals that spend all or part of their lives in water. They play a critical role in aquatic ecosystems. It is an integral part of the aquatic food web. By eating leaves, algae and bacteria in the environment, they convert organic matter into a food source that fish and other vertebrates can use. Fish are considered highly effective top predators for aquatic creatures in the food web. Fish are known to have strong effects on the abundance, species richness and community structure of macroinvertebrates and constitute the primary diet of many of them. For this reason, the number of insects is much higher in ponds and coastal vegetation where there are no fish. Coastal vegetation can play a very important role as a refuge for invertebrates that are vulnerable to predation. In this study, the roles and importance of benthic macroinvertebrates in the food web, which are found in increasingly decreasing water resources today, are examined.

Keywords: Benthic macroinvertebrate, Food web, Water resources

INTRODUCTION

Aquatic macroinvertebrates live in all types of freshwater environments, from pristine mountain streams to wetlands and sewage ponds. If a few rocks in shallow water in any healthy pond or stream are turned over, many water bugs can be seen. Larvae or nymphs of well-known flying insects such as mayflies and dragonflies are found. Some creatures, such as freshwater snails and mussels, spend their entire lives in water. Creatures such as dragonflies and mosquitoes live in water in their larval and pupal stages, but outside of water in their adult stages (URL 1; 2). They are often found attached to rocks, vegetation, logs and branches, or buried in bottom sand and sediments. They are reliable bioindicators because they spend all or most of their lives in water, are easy to collect, and have different tolerances to pollution (URL 3). Because they cannot escape pollution and are relatively immobile, unlike fish, they have the capacity to integrate the effects of stress factors over time (URL 2; 3).

The Importance of Benthic Macroinvertebrates in Aquatic Life

Macroinvertebrates are very important for aquatic ecosystems. They are food for vertebrates and their biodiversity is a testament to the quality of their habitat (Brysiewicz et al., 2022). Assessing the abundance and diversity of benthic macroinvertebrates in a water body provides an insight into the biological status of the water body. In general, water bodies in healthy biological state support a wide variety and high numbers of macroinvertebrate taxa, including those intolerant to pollution. Areas showing only pollution-tolerant species or little diversity or abundance indicate a less healthy water body. Biological status is the most comprehensive indicator of water body health. When the biology of a water body is healthy, the chemical and physical components of the water body are generally in good condition as well (URL 2, 6).

Aquatic macroinvertebrates play important roles in many ecological processes in aquatic ecosystems. Breakdown of leaf litter occurs through the presence of invertebrates that cut or chew leaf material (Wallace et al., 1982; Cuffney et al., 1990). Especially in small streams, the amount of defoliation generally increases when the numbers of invertebrates decrease (Webster and Benfield, 1986). In addition, macroinvertebrates that consume algal resources also have significant effects on algae in terms of biomass and primary production (Lamberti and Resh, 1983). Grazing macroinvertebrates is also beneficial as they recycle nutrients into the environment (Vanni, 2002). As primary consumers of aquatic foods, macroinvertebrates form an important link at trophic levels (e.g. fish) in networks between primary resources (e.g. algae and detritus) and higher-order resources (Díaz Villanueva et al., 2012). However, their role is not only to provide energy to upper trophic levels, but also to regulate energy flow through aquatic food webs (Wallace and Webster, 1996).

Classification of Benthic Macroinvertebrates According to Their Feeding Types

Each taxon has its own specific role in the ecosystem determined by the way it feeds. Scientists can categorize benthic macroinvertebrates into groups called functional feeding groups. Taxa in similar functional feeding groups are often found in similar habitats. Some taxa prefer fast-moving riffles, others prefer slow-moving pools or other habitat. A complex and healthy ecosystem supports a variety of functional feeding groups. Macroinvertebrates are divided into four classes according to their morphological (e.g. mouth part specialization) and behavioral characteristics, according to the mechanisms they use when consuming resources (e.g. feeding style) (Ramírez et al., 2014).

♦ Scrapers get food by scraping algae off rocks and other hard surfaces in the stream channel. It lives in parts of streams where sunlight can reach the bottom to allow algal growth. These areas are often in shallow, fast-moving riffles. As a result, many scrapers have adapted to stay attached to rocks in fast currents. Scrapers may include snails, certain mayfly larvae, and limpets (Lock et al., 1984).

♦ Shredders feed on leaves, twigs, and other pieces of organic matter that fall into a stream. They need lots of trees overhead (a dense canopy cover) to supply these materials. They chew up debris into smaller particles These smaller particles then become a source of food for Collectors. Shredders include stonefly larvae and scuds (Ramírez et al., 2014).

♦ Collectors eat fine organic matter. This includes leaf fragments, bacteria, fine sediment, and waste products from other organisms. There are two categories of collectors: filtering and gathering. Filtering collectors feed by filtering food out of passing water. They do this with hair-like fans or by spinning a fine net. Gathering collectors feed by gathering food from the bottom. Collectors are most common in slower-moving, low gradient streams where sediments collect. Even so, Collectors live in all types of stream reaches. Collectors include worms, blackfly larvae, and many mayfly and caddisfly larvae and midges (Hershey, 1987).

♦ Predators eat other macroinvertebrates and even fish. Predators can live anywhere in a stream but are less abundant than other feeder types. There must be enough prey to support predators, or they will not be present at all. Predators include the larvae of dragonflies, damselflies, and dobsonflies, among others. Adult aquatic beetles are predators. The larvae of some beetles, flies, stoneflies, caddisflies, and mayflies can be predators. But those taxa contain other functional feeding groups as well (Ramírez et al., 2014).

The Importance of Benthic Macroinvertebrates in the Freshwater Food Web

The habitats of macroinvertebrates are rocks and sediments at the bottom of the stream, plants in the water and around the stream, leaf litter and decomposed organic matter falling into the stream, and submerged logs, branches, woody debris. Macroinvertebrates need shelter and nutrition in these habitats. In choosing the best shelter, they tend to congregate in areas that provide the most food and the most dissolved oxygen.

Benthic macroinvertebrates, an integral part of the aquatic food web, transform energy stored in the organic environment. They eat leaves, algae, and bacteria, and are in turn eaten by fish, amphibians, birds, and other vertebrates. Thus, they transform the substance in the aquatic environment into a food source that fish and other vertebrates can use. When benthic macroinvertebrates die, the cycle repeats, releasing nutrients to be reused by aquatic plants and animals (URL 4; 6).

Macroinvertebrates are an important food source for other aquatic animals. Aquatic insects help recycle nutrients from the bottom of ponds or streams into the food web. Young life stages, often called larvae or nymphs, eat algae and waste that settle at the bottom of the water. Many other animals feed on aquatic insects. Fish take aquatic insect larvae and nymphs as food along the pond or stream bed. Some fish catch adult insects from the water surface or jump to the water surface to eat flying adult insects. Birds and amphibians may eat aquatic and flying insect adults. If the macroinvertebrate community does not develop, the nutritional impact on all animals in the ecosystem that depend on it will be felt as a problem (URL 5).

The function of specific macroinvertebrates can best be understood by describing their functions in their ecosystems. Classic examples include the role of decomposer insects (e.g. facilitate the decomposition of leaves) in contributing to the decomposition processes of organic matter (Cummins et al., 1989), as well as in the production of fine particles that are carried downstream, deposited at the bottom and used by other consumers such as foragers (Cummins and Klug, 1979). Others provide downward spiraling by controlling primary production, detritus breakdown, and nutrient mineralization.

Over the past few decades, changes in land use through the conversion of global forest cover to exotic plantation are contributing to both habitat and biodiversity loss and species extinction. The richness and abundance of macroinvertebrates are clearly evident in streams dominated by natural riparian vegetation. Living things are at higher rates in natural forest areas than in areas with exotic vegetation. Collectors are the most abundant functional feeding group here. Their availability varies in different parts of the stream.

Vegetation, dead leaves, and large woody debris at the edge of headwaters of forest areas are an important source of energy and nutrients for stream food webs (Vannote et al., 1980). In these environments, the relatively high water velocity and wide shade of the canopy provide autochthonous production (Vannote et al., 1980; Wallace et al., 1997). Therefore, changes in coastal vegetation can alter the quality of leaf litter inputs in aquatic communities and affect processes such as trophic structure and composition in aquatic ecosystems (Abelho and Graça, 1996; Martínez et al., 2013).

Several studies conducted in headwaters show higher fragment richness, higher invertebrate density and richness in streams dominated by native forests compared to streams dominated by exotic patches in other forests. Because macroinvertebrate communities represent intermediate trophic links between primary and tertiary consumers as sources of fish feed (Bertrán et al., 2013; Cornejo-Acevedo et al., 2014; Fierro et al., 2014; Fierro et al., 2015; Jensen et al., 2012).

Low to moderate populations of macroinvertebrates can affect the fish-carrying capacity of streams. If the availability of prey is limited, this will affect predatory fish (Pequeno et al., 2010). Therefore, changes in any macroinvertebrate community cause changes in the functioning of aquatic ecosystems and restructuring of food chains (Richards et al., 1996; Tiziano et al., 2014).

Fish are known to have strong effects on invertebrate abundance, species richness and community structure. Coastal vegetation can play a crucial role as a refuge for invertebrates vulnerable to fishing. The responses and mesohabitat preferences of macroinvertebrate taxa in coastal and open waters in the presence of fish do not show any differences. On the contrary, in fish-free waters, the total density of aquatic insects in the coastal vegetation is much higher in number and richer in terms of species. They prefer coastal areas in non-significantly higher numbers in waters containing fish, but species richness is independent.

Predator–prey relationships between fish and aquatic macroinvertebrates are crucial in structuring freshwater communities (Bendell and McNicol, 1995; Batzer et al., 2000). Fish are considered highly effective top predators of aquatic organisms in the food chain, and many insect species are their primary food (Kottelat and Freyhof, 2007). Studies involving manipulation of fish presence or abundance have yielded dramatic results on changes in the density, species composition, and size structure of macroinvertebrate communities (Leppä et al., 2003; Nieoczym et al., 2020). Many macroinvertebrate taxa are virtually absent from fish-dominated waters (Nieoczym and Kloskowski, 2015; Toro et al., 2020). Fish also have a strong influence on the distribution of macroinvertebrates within water bodies (Schilling et al., 2009). However, species with defensive adaptations can survive in the presence of fish (Pope and Hannelly, 2013).

Many species of fish use vegetation near shore to reduce predator pressure. They are also found in the open water zone when fish are not available (Kloskowski et al., 2020). However, species with antipredators, such as the secretion of toxic substances, and those with morphological features for adaptation or defense against fish do not need shelters such as a habitat containing vegetation (Nieoczym et al., 2023).

Interactions between fish and invertebrates in large water bodies consisting of different mesohabitats are affected by the presence of complex structured vegetation character of streams (Kajgrova et al., 2021). Vegetation affects abiotic parameters such as increased habitat and resource diversity in waters, depth of light penetration, temperature, dissolved organic matter and oxygen concentration in water (Lürig et al., 2021). In habitats with complex macrophyte structure, the hunting efficiency of fish is higher than in open waters where there is no or sparse vegetation (de Mendoza et al., 2012). In natural environments, prey within vegetation is much less conspicuous to predatory fish. Moreover, dense vegetation harms fish and reduces the predator's ability to move and track its prey (Wirsing et al., 2010). Many studies have reported that aquatic macroinvertebrates, including Odonata (larvae), Heteroptera and Coleoptera, prefer coastal habitats rich in vegetation that is believed to protect them and even restrict their distribution against predators (Tolonen et al., 2003).

Using Macroinvertebrates as Bioindicators in Aquatic Environments

Aquatic insects tell a lot about ecosystems–in other words, they are bioindicators. Scientists use aquatic insects to check the health of pond and stream ecosystems. Because some macroinvertebrates are very sensitive to poor water quality and human impacts. It is sometimes difficult to find ways to quantitatively measure overall ecosystem health. Ecosystem health depends on many factors (e.g. temperature, nutrient cycling, water chemistry). Scientists create biological integrity indices to measure water quality. Indices are basically an overall health score based on a community of ecosystems. The health of the aquatic ecosystem is a number that looks at the richness of EPT: different species of Ephemeroptera, Plecoptera and Trichoptera.

Healthy ecosystems have many different species of aquatic insects and no group of creatures that dominates the system. Generally, the presence of different species means a healthier ecosystem. An unhealthy pond or stream ecosystem cannot support a large and especially diverse population of water insects. Some macroinvertebrates are sensitive to pollution, while others can survive in very polluted waters. Often dirty water contains creatures such as worms, daphnia and non-biting midge larvae. In waterways that are degraded and subject to eutrophication (nutrient pollution), there are large amounts of sandflies, Oligochaeta and mosquitoes that disturb the surrounding residents.

Organisms in clean waters are completely intolerant to pollution. These are: Mayfly nymphs, Stonefly nymphs Caddisfly larvae Hellgrammites (dobsonfly larvae) Gilled snails Water pennies Adult riffle beetles. To survive, they need the cleanest water with high levels of dissolved oxygen. In particular, mayflies (Ephemeroptera), stoneflies (Plecoptera) and caddisflies (Trichoptera) are the most sensitive to changes in water quality. The percentage of "EPT" taxa in the aquatic habitat of a stream is an indicator of clean water and well-being. Moderately pollution tolerant: Dragonfly nymphs, Damselfly nymphs, Alderfly larvae, Crane fly larvae, Scuds, aquatic sowbugs Crayfish Freshwater clams, Freshwater mussels. Groups that are tolerant to pollution: Blackfly larvae, Midge larvae, Leeches, Aquatic worms, Lunged snails. They can survive in areas with poor water quality and low dissolved oxygen levels.

The basic principle behind studies on macroinvertebrates is that some are more sensitive to pollution than others, so they are more numerous. Therefore, if a stream site is inhabited by organisms that can tolerate pollution and the more pollution-sensitive organisms there are, a pollution problem is likely to occur.

Therefore, if a stream site is inhabited by organisms that can tolerate pollution and the more pollution-sensitive organisms there are, a pollution problem is likely to occur. If a biological survey shows that stoneflies are absent from the area, the amount of dissolved oxygen has fallen below the point to protect them, which may have prevented them from reproducing or killed them completely. Other factors may also contribute to their extinction, such as pollutants discharged from factories or habitat degradation such as misuse of agricultural land, very high water temperatures, and excessive sand or silt accumulation on stream bottoms (Ramírez et al., 2014).

DISCUSSION AND CONCLUSIONS

Reproductive success and predation risk are assumed to be important factors responsible for the distribution of many aquatic macroinvertebrates across water bodies (Nicolet et al., 2004; Gioria, 2014). Aquatic insect densities and species richness are lower in ponds with fish than in ponds without fish. However, some data show that aquatic insects living in fish-dominated waters can sustain the presence of parasites or predators in food webs (Proctor et al., 2015) and possibly even benefit from the presence of fish (Nieoczym et al., 2023; Pope and Hannelly, 2013).

Coastal habitat is also important for aquatic macroinvertebrates (Burks et al., 2002). Aquatic insects are found in higher abundances in coastal vegetation than in open water areas. Spatial distribution enables many macroinvertebrates to avoid predators other than fish. Aquatic insects are found in high densities in coastal mesohabitats where fish are absent. Large-sized predatory invertebrates, including predatory congeners whose habitat structure attacks their prey by ambush, are a significant threat to smaller species/individuals (Klecka and Boukal, 2014). On the other hand, in the absence of fish, invertebrate predators such as large-sized Dytiscidae may move out of the coastal habitat to feed (Kloskowski et al., 2020). Therefore, small-sized macroinvertebrates prefer the dense vegetation of coastal habitats rather than open water areas to protect themselves from predators, unless there is a morphological or chemical difference (Nieoczym et al., 2023).

Altering or disrupting the ecosystem affects the food chain by causing the loss of existing benthic species. The consequences of each species loss on the food web are unpredictable. If species are disappearing from an ecosystem, probably one after the other, at some point, there are major changes in the ecosystem. When species disappear, extremely expensive costs arise from engineering processes to preserve natural ecosystems. In theory, ecosystem processes are expected to continue when at least one species from each functional group remains and the process rate is sufficient (Covich et al., 1999).

Freshwater habitats have been significantly damaged in the last few decades. With the call for ecological integrity of the European Water Framework Directive, biodiversity has increased especially in aquatic environments. Although Lake Erie was once dead, conservation now increases dissolved oxygen levels and mayflies in bottom water sediments, resulting in the increase of many fish species (Covich et al., 1999; Nieoczym et al., 2023).

Water quality in rivers and indirectly macrozoobenthos communities are affected by agricultural land use in their catchments. Changes in environmental conditions as a result of land use can lead to reductions in biodiversity, biomass, and density of sensitive species of aquatic animals and thus changes in biotic composition. Small aquatic ecosystems are particularly sensitive to human pressures (Wohl, 2017).

REFERENCES

Abelho, M., M. A. S. Graça. 1996. Effects of Eucalyptus Afforestation on Leaf Litter Dynamics and Macroinvertebrate Community Structure of Streams in Central Portugal. Hydrobiologia 324: 195–204.

Batzer, D. P., C. R. Pusateri, R. Vetter. 2000. Impacts of Fish Predation on Marsh Invertebrates: Direct and Indirect Efects. Wetlands, 20: 07–312. https://doi.org/10.1672/0277-5212(2000) 020[0307:IOFPOM]2.0.CO;2

Bendell, B. E., D. K. McNicol. 1995. Lake Acidity, Fish Predation, and the Distribution and Abundance of Some Littoral Insects. Hydrobiologia 302: 133–145. https://doi.org/10.1007/BF00027038

Bertrán, C., C. Jiménez, P. Fierro, F. Pena-Cortés, J. Tapia, E. Hauenstein, L. Vargas-Chacoff. 2013. Alimentación de Micropogonias Furnieri (Osteichthyes: Sciaenidae) en el Lago Costero Budi, Sur de Chile. Rev. Biol. Mar., 48: 193–197.

Brysiewicz, A., P. Czerniejewski, J. Dąbrowski, K. Formicki. 2022. Characterisation of Benthic Macroinvertebrate Communities in Small Watercourses of the European Central Plains.

Burks, R. L., D. Lodge, E. Jeppesen, T. Lauridsen. 2002. Diel Horizontal Migration of Zooplankton: Costs and Benefts of Inhabiting the Littoral. Freshwater Biology, 47: 343–365. https://doi.org/10.1046/j.1365- 2427.2002.00824.x

Cuffney, T. F., J. B. Wallace, G. J. Lugthart. 1990. Experimental Evidence Quantifying the Role of Benthic Invertebrates in Organic Matter Dynamics of Headwater Streams. Freshwater Biology, 23: 281-299.

Cummins, K. W., M. J. Klug. 1979. Feeding Ecology of Stream Invertebrates. Annual Review in Ecology and Systematics, 10: 147-172.

Cummins, K. W., M. A. Wilzbach, D. M. Gates, J. B. Perry, W. B. Taliaferro. 1989. Shredders and Riparian Vegetation: Leaf Litter that Falls into Streams Influences Communities of Stream Invertebrates. *BioScience,* 39(1): 24–30. https://doi.org/10.2307/1310804

Cornejo-Acevedo, M. F., P. Fierro, C. Bertrán, L. Vargas-Chacoff. 2014. Composición y Sobreposición Dietaria de Pinguipes Chilensis (Perciformes, Pinguipedidae), Cheilodactylus Variegatus (Perciformes, Cheilodactylide) y Aplodactylus punctatus (Perciformes, Aplodactylidae) en el Litoral Costero Valdiviano, Chile. Gayana, 78: 123–133.

Covich, A. P., M. A. Palmer, T. A. Crowl. 1999. The Role of Benthic Invertebrate Species in Freshwater Ecosystem. Bioscience, 49(2): 119-127. https://doi.org/10.2307/1313537

de Mendoza, G., E. Rico, J. Catalan. 2012. Predation by Introduced Fish Constrains the Thermal Distribution of Aquatic Coleoptera in Mountain Lakes. Freshwater Biology, 57: 803–814. https://doi.org/10. 1111/j.1365-2427.2012.02746.x

Díaz Villanueva, V., R. Albariño, C. Conhoto. 2012. Positive Effect of Shredders on Microbial Biomass and Decomposition in Stream Microcosms. Freshwater Biology, 57(12): 2504–2513. Doi: 10.1111/fwb.12023

Fierro, P., C. Bertrán, D. Martínez, C. Valdovinos, L. Vargas-Chacoff. 2014. Ontogenetic and Temporal Changes in the Diet of the Chilean Silverside Odontesthes Regia (Atherinidae) in Southern Chile. Cahiers de Biologie Marine, 55: 323–332.

Fierro, P., C. Bertrán, M. Mercado, F. Pena-Cortés, J. Tapia, E. Hauenstein, L. Caputo, L. Vargas-Chacoff. 2015. Landscape Composition as a Determinant of Diversity and Functional Feeding Groups of Aquatic Macroinvertebrates in Southern Rivers of the Araucanía. Chile Lat. Am. J. Aquat. Res., 43: 186–200.

Gioria, M. 2014. Habitats. In: Yee DA (Ed) Ecology, Systematics, and the Natural History of Predaceous Diving Beetles. Dytiscidae Coleoptera, 307–362.

Hershey, A. E. 1987. Tubes and Foraging Behavior in Larval Chironomidae: Implications for Predator Avoidance. Oecologia, 73: 236-241.

Jensen, H., M. Kiljunen, P. A. Amundsen. 2012. Dietary Ontogeny and Niche Shift to Piscivory in Lacustrine Brown Trout *Salmo trutta* Revealed by Stomach Content and Stable Isotope analyses. Journal of Fish Biology, 80: 2448–2462.

Kajgrova, L., Z. Adamek, J. Regenda, C. Bauer, V. Stejskal, O. Pecha, D. Hlavac. 2021. Macrozoobenthos Assemblage Patterns in European Carp (Cyprinus carpio) Ponds – the Importance of Emersed Macrophyte Beds. Knowledge and Management of Aquatic Ecosystems, 422: 9. https:// doi.org/10.1051/kmae/2021008

Klecka, J., D. S. Boukal. 2014. The Efect of Habitat Structure on Prey Mortality Depends on Predator and Prey Microhabitat Use. Oecologia, 176: 183–191. https://doi.org/10.1007/s00442-014-3007-

Kloskowski, J., M. Nieoczym, R. Stryjecki. 2020. Between-Habitat Distributions of Pond tadpoles and Their Insect Predators in Response to Fish Presence. Hydrobiologia, 847: 1343–1356. https://doi.org/ 10.1007/s10750-020-04190-5

Kottelat, M., J. Freyhof. 2007. Handbook of European Freshwater Fishes. Publications Kottelat.

Lamberti, G. A., V. H. Resh. 1983. Stream Periphyton and Insect Herbivores: An Experimental Study of Grazing by a Caddisfly Population. Ecology, 1124-1135. https://doi.org/10.2307/1937823

Leppä, M., H. Hämäläinen, J. Karjalainen. 2003. The Response of Benthic Macroinvertebrates to Whole-Lake Biomanipulation. Hydrobiologia, 498: 97–105. https://doi.org/10.1023/A:1026224923481

Lock, M., J. Costerton, R. Ventullo, R. Wallace, S. Charlton. 1984. River Epilithon: toward a Structuralfunctional Model. Oikos, 42: 10-22.

Matthews, B. 2021. Submerged Macrophytes Afect the Temporal Variability of Aquatic Ecosystems. Freshwater Biology, 66: 421–435. https://doi.org/10.1111/fwb.13648

Martínez, A., A. Larranaga, J. Pérez, E. Descals, A. Basaguren, J. Pozo. 2013. Effects of Pine Plantations on Structural and Functional Attributes of Forested Streams. Forest Ecology and Management, 310: 147–155.

Nicolet, P., J. Biggs, G. Fox, M. J. Hodson, C. Reynolds, M. Whitfeld, P. Williams. 2004. The Wetland Plant and Macroinvertebrate Assemblages of Temporary Ponds in England and Wales. Biological Conservation, 120: 261–278. https://doi.org/10.1016/j.biocon.2004.03.010

Nieoczym, M., J. Kloskowski. 2015. Responses of Epibenthic and Nektonic Macroinvertebrate Communities to a Gradient of Fish Size in Ponds. Journal of Limnology, 74: 50–62. https://doi.org/10.4081/jlimnol. 2014.981

Nieoczym, M., R. Mencfel, M. Gorzel, J. Kloskowski. 2020. Reduced Abundance But Increased Diversity of Chironomid Larvae under Higher Trophic Pressure from Fish in Semi-Permanent Ponds. Limnologica, 82: 125778. https://doi.org/10.1016/j.limno.2020. 125778

Nieoczym, M., R. Stryjecki, P. Buczyński, W. Płaska, J. Kloskowski. 2023. Diferential Abundance, Composition and Mesohabitat Use by Aquatic Macroinvertebrate Taxa in Ponds with and without Fisher Aquatic Sciences, 85: 25. https://doi.org/10.1007/s00027-022-00922-y

Pequeno, G., H. Pavés, C. Bertrán, L. Vargas-Chacoff. 2010. Seasonal Limnetic Feeding Regime of the Robalo Eleginops Maclovinus (Valenciennes 1830), in the Valdivia River, Chile. Gayana, 74: 47–56.

Pope, K. L., E. C. Hannelly. 2013. Response of Benthic Macroinvertebrates to Whole-Lake, Non-Native Fish Treatments in Mid-Elevation Lakes of the Trinity Alps, California. Hydrobiologia, 714: 201–215. https://doi.org/10.1007/s10750-013-1537-2

Proctor, H. C., I. M. Smith, D. R. Cook, B. P. Smith. 2015. Subphylum Chelicerata, Class Arachnida. In: Thorp JH, Rogers DC (Eds) Ecology and General Biology: Thorp and Covich's freshwater invertebrates, 4th edn. Academic Press, 599–660. https://doi.org/ 10.1016/B978-0-12-385026-3.00025-5

Ramírez, A., P. E. Gutiérrez-Fonseca. 2014. Functional Feeding Groups of Aquatic Insect Families in Latin America: A Critical Analysis and Review of Existing Literature. Revista de Biologia Tropical, 62(2): 155-167.

Richards, C., L. B. Johnson, G. E. Host. 1996. Landscape-Scale Influences on Stream Habitats and Biota. Canadion Journal of Fisheries and Aquatic Sciences, 53: 295–311.

Schilling, E. G., C. S. Loftin, A. D. Huryn. 2009. Macroinvertebrates as Indicators of Fish Absence in Naturally Fishless Lakes. Freshwater Biology, 54: 181–202. https://doi.org/10.1111/j.1365-2427.2008.02096.x

Tiziano, B., M. Cammarata, M. J. López-Rodríguez, J. M. Tierno de Figueroa, M. Baltieri, P. Varese, S. Fenoglio, 2014. The Influence of Water Quality and Macroinvertebrate Colonization on the Breakdown Process of Native and Exotic Types in Sub-Alpine Stream. Journal of Freshwater Ecology, 29: 159–169.

Tolonen, K., H. Hämäläinen, I. Holopainen, K. Mikkonen, J. Karjalainen. 2003. Body Size and Substrate Association of Littoral Insects in Relation to Vegetation Structure. Hydrobiologia, 499: 179–190. https://doi.org/10.1023/A:1026325432000

Toro, M., I. Granados, Á. Rubio, A. Camacho, I. Sabás, M. Ventura, C. Montes. 2020. Response of the Aquatic Invertebrate Community to the Eradication of an Exotic Invasive Fish 30 Years after Its Introduction into an Iberian Alpine Lake. Aquatic Sciences, 82: 55. https://doi.org/ 10.1007/s00027-020-00728-w

URL 1. Aquatic Macroinvertebrates (https://www.sciencelearn.org.nz/resources/1820-freshwater-macroinvertebrates)

URL 2. EPA United States Environmental Protection Agency (https://www.epa.gov/national-aquatic-resource-surveys/indicators-benthic-macroinvertebrates#:~:text=Benthic%20macroinvertebrates%20are%20commonly%20used,in%20their%20tolerance%20to%20pollution.).

URL 3. The importance of macroinvertebrates in freshwater streams. (https://habitat.fisheries.org/the-importance-of-macroinvertebrates-in-freshwater-streams/).

URL 4. What in the World are Benthic Macroinvertebrates? (https://www.iihr.uiowa.edu/igs/publications/uploads/wfs-2010-07.pdf)

URL 5. Aquatic macroinvertebrates (https://www.wa.gov.au/service/natural-resources/water-resources/aquatic-macroinvertebrates)

URL 6. What are benthic macroinvertebrates? https://kingcounty.gov/en/dept/dnrp/nature-recreation/environment-ecology-conservation/science-services/benthic-).

Vanni, M. J. 2002. Nutrient Cycling by Animals in Freshwater Ecosystems. Annual Review of Ecology. Evolution, and Systematics, 33, 341-370. https://doi.org/10.1146/annurev.ecolsys.33.010802.150519

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, C. E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences, 37: 130-137.

Wallace, J. B., S. L. Eggert, J. L. Meyer, J. R. Webster. 1997. Multiple Trophic Levels of a Forest Stream Linked to Terrestrial Litter Inputs. Science, New Series, 277(5322): 102-104. Doi: 10.1126/science.277.5322.102

Wallace, J. B., J. R. Webster. 1996. The Role of Macroinvertebrates in Stream Ecosystem Function. Annual Review of Entomology, 41(1): 115-139. Doi: 10.1146/annurev.ento.41.1.115

Wallace, J. B., J. R. Webster, T. F. Cuffney. 1982. Stream Detritus Dynamics: Regulation by Invertebrate Consumers. Oecologia (Berlin), 53: 197-200.

Webster, J. R., E. F. Benfield. 1986. Vascular Plant Breakdown in Freshwater Ecosystems. Annual Review ol Ecology Systematics, 17: 567-594.

Wilson, E. O. 1992. The Diversity of Life. New York: W.W. Norton.

Wirsing, A. J., K. E. Cameron, M. R. Heithaus. 2010. Spatial Responses to Predators Vary with Prey Escape Mode. Animal Behaviour, 79: 531–537. https://doi.org/10.1016/j.anbehav.2009.12.014

Wohl, E. 2017. The Significance of Small Streams. Frontiers Earth Science, 11: 447–456.

GREEN BUILDINGS FOR SUSTAINABLE DEVELOPMENT

Nurcan OZKAN[1]

[1] *Trakya University, Education Faculty, Mathematics and Sciences Education Department, Edirne, Turkey*
Corresponding author e-mail: nurcanozkan@hotmail.com

ABSTRACT

In this study, a general evaluation of the green building concept, its importance, benefits and certification systems, which is evaluated within the concept of sustainability and whose importance has been increasing in the world in recent years, has been made. Among the most important reasons for the increase in global warming, climate changes, increasing environmental pollution, rapid decrease in natural resources and depletion of energy resources in the world are buildings produced with traditionally built building construction technology. In today's conditions, environmental protection has become more important in housing construction, and interest in new environmentally friendly buildings, called green buildings, has increased. The concept of green building has started to develop all over the world and in Europe in recent years, new institutions have been established in this direction, new certification programs have been created and the concept of green building has started to spread in all countries. The most important features of green buildings are that they contribute to a healthier environment in the future by ensuring that buildings, which have a significant impact on environmental and air pollution, are sustainable. Green buildings are buildings that respect nature, are healthy, comfortable, ecological and can use renewable energies. Green, ecological, climate and environment friendly, zero carbon emission, high performance buildings are rapidly becoming part of our agenda. With the spread of the green building concept, developed countries have developed certification systems to evaluate and classify the environmental impacts of buildings that require certain standards related to sustainable buildings. Certification systems generally evaluate buildings based on several parameters, such as the existence of systems to reduce energy and water consumption, the environmental friendliness of the building materials used, lighting, air quality and comfort related to user comfort within the building, and buildings are certified in this way.

Keywords: Sustainability, Green building, Green building certification systems, Environmentally friendly

INTRODUCTION

It is known that residential buildings constitute the majority of world energy consumption. An important issue that causes great energy waste in these buildings is that, in addition to inefficient consumption habits, the buildings are produced with conventional construction technology (Anbarcı et al., 2012; Benedict and McMahon, 2001; Burnett, 2007; Kaylı and Güneş Gölbey, 2020).

Global warming, thirst, environmental pollution and rapid consumption of natural resources have brought the construction of environmentally friendly, ecological buildings to the agenda in the construction sector. As interest in environmentally friendly building construction increases, structures called green buildings have emerged. Green buildings, which are certified by introducing certain standards, have created a new trend and sector in the construction industry as buildings that are more valuable, respectful to nature, ecological, comfortable and reduce energy consumption (Anbarcı et al., 2012; Doğan and Seçme, 2018).

Sustainable architecture; It is defined as a set of activities in which the damage to nature is minimized during the construction, use and demolition processes of needed buildings, ecological balance is observed, and materials, water and energy are used effectively. With sustainable buildings, it is aimed to protect the health and comfort of the users, not to endanger the existence and future of natural resources during the construction and use phases, and to create waste in a way that does not harm nature (Geçer et al., 2019; Gür, 2007; Yılmaz et al., 2016). Green building evaluation systems and certification programs have been created to concretely determine the negative effects of buildings on the environment (Erten, 2011; Hoşgör, 2014; Yücel Işıldar and Gökbayrak, 2018).

Housing Sector and Green Building Concept within the Scope of Sustainability

The rapid increase in the demand for natural resources and energy as a result of population growth, industrialization, rapid advancement in technology and globalization in the world, and the decrease in non-renewable energy resources direct the society to the use of renewable energy resources. In this context, the concept of sustainability has entered the lives of human beings in order to leave a healthy world to future generations (Saka, 2011).

Buildings have extensive direct and indirect impacts on the environment. Buildings; They use energy, water and raw materials, produce waste and emit potentially harmful atmospheric emissions during construction, use, renovation, re-use and demolition processes. These environmental impacts have necessitated the creation of green building standards, certifications and rating systems aimed at reducing the impact of buildings on the natural environment through sustainable design.

Green building; It is a construction method that produces healthier buildings with less negative impact on the environment, lower maintenance costs, and uses resources efficiently. The term green building, also known as sustainable building, high performance building and green construction; are used interchangeably to describe essentially the same thing.

Green buildings; It is designed with a holistic approach and an understanding of social and environmental responsibility, starting from the land selection of the building and evaluating it within the framework of its life cycle. In addition, they are defined as sustainable structures that are compatible with climate data and local conditions, consume as much as they need, are oriented towards renewable energy sources, use natural and waste-free materials, encourage participation, are sensitive to ecosystems (Sur, 2012). Any building, home, office, school, hospital, community center can be a green building provided that it includes green building features (URL 1).

While green buildings protect and improve the health and productivity of users with natural light and good indoor air quality, they are sensitive to the consumption of natural resources during construction and use, do not cause environmental pollution, create resources for other structures after demolition, or return to their place in nature without harming the environment are structures (Sev, 2009).

Why Should We Go Green?

Buildings are a tremendous source of carbon emissions. They are also places where there is significant room for improvement. Many people are realizing that when going green they can reduce their carbon footprint and actually give the environment a helping hand.

Whether it is constructed, purchased or rented, it is necessary to minimize personal emissions as much as possible in today's conditions. A green home often means financial savings and improved quality of life. And it all starts with a decent-sized house in a decent location.

The construction industry is thinking about how to build more sustainable buildings. Architects and engineers work on designs that will cause minimum harm to nature. The traditional building construction process creates problems, especially regarding energy efficiency, and uninsulated buildings lead to more energy consumption. When green building is mentioned, many processes are encountered such as making the raw materials used more sustainable, the efficiency of the construction process, and minimizing the waste generated in the construction of buildings.

Green buildings, in addition to playing an active role in sustainable architecture, construction and environmental protection, also provide many benefits to their buyers and all other people. Despite lower user and maintenance costs, it offers more comfort, healthier quality of life and longer-lasting use.

Green buildings are buildings that cause less harm to the environment compared to traditional construction practices. Innovative technologies and materials are used in their construction. Insulated construction materials are preferred, thus saving on heating and cooling costs. Rainwater can be collected. It is designed to benefit from renewable energy sources. Some green buildings are built integrated with solar and wind panels. Some buildings can be covered with plants to reduce carbon emissions. The land on which the building is located is utilized at maximum efficiency (URL 1).

What are the Features of Green Buildings?

• Green buildings are buildings that are compatible with the environment and cause minimal damage to nature. When it comes to green buildings, first of all, efficient buildings are mentioned. Insulated buildings reduce energy consumption and increase energy efficiency. However, energy efficient buildings do not only use insulated materials.

• 'Solar heat gain coefficient' is taken into consideration when constructing buildings. In its simplest form, this is the name given to the sunlight entering through the glass. The more sunlight entering a building, the hotter the building becomes. This also reduces energy costs. Buildings that can make maximum use of sunlight also reduce lighting costs. By a similar logic, the construction of airtight walls in building construction keeps moisture out and reduces cooling costs by making the building cooler in the summer months.

• Mechanisms that use water efficiently are also preferred in the construction of green buildings. Siphons and taps that use water efficiently are installed.

• Solar panels and wind turbines are used when constructing these buildings. The energy obtained is stored and used in the energy expenses of the building. In this way, buildings both contribute to the environment and save energy (Figure 1).

• When constructing buildings, materials that do not harm the environment are preferred. Thus, toxic waste is reduced to a minimum. The use of sustainable materials compatible with nature both reduces environmental pollution and contributes to the protection of biodiversity.

• Some green buildings are built with technologies that can harvest rainwater. The captured water is filtered and used within the building. In this way, water costs are also saved.

• There are recycling facilities inside green buildings. Biological waste is converted into compost and fertilizer.

• Most green buildings are constructed so that plants can grow on their exterior walls (Picture 2). These plants both absorb rainwater and contribute to the prevention of air pollution by reducing the effects of carbon monoxide emissions (URL 2).

Figure 1. Green buildings for power plant and sports facility use, CopenHill (Copenhagen, Denmark) (URL3).

Figure 2. Green buildings used for residential purposes with plants grown on their exterior walls (URL 4).

The Increasing Importance of Green Buildings

Buildings are a significant source of carbon emissions. They are also places where there is significant room for improvement. Many people realize that by going green they can reduce their carbon footprint and actually give the environment a helping hand (Figure 3).

Considering the increasing world population and increasing urbanization, the contributions of the construction industry for a sustainable future are becoming invaluable. The increasing importance of green buildings shows that not only states but also private institutions have an important role to play in the construction of a sustainable future.

Figure 3. Example of multi-purpose green buildings (URL 5).

After the industrial revolution, construction focused on supply rather than sustainability. Rapidly increasing demand, especially in the years following the Second World War, brought about rapid urbanization. In the light of scientific research, it is known that concrete is a major contributor to CO_2 emissions. Increasing social, individual and corporate awareness has made a great contribution to the design of sustainable buildings, the rethinking of sustainable construction and the formation of sustainable cities.

It is certain that the terms 'sustainable urbanization' and 'green building' will be heard frequently in the coming years. Technology is gradually developing. Reducing the negative effects of climate change due to global warming and reducing carbon emissions is only possible with a collective effort. An important part of building environmentally compatible lives will undoubtedly be green buildings (URL 2).

What are Green Building Standards?

Each of the green building energy certification systems, which emerged after the decisions and sanctions made by some world states after the industrial revolution, were initially created to serve their own countries. Some of the emerging certification systems have, over time, turned into green building certification systems that are valid worldwide. The prominent ones can be listed as BREEAM, LEED and DGNB.

a. Breeam Green Building Certification Systems

Breeam (The Building Research Establishment's Environmental Assessment Method) certification system is the first green building assessment system developed in England in 1990. For this reason, it is recognized as the most trusted certification system today (Breeam, 2011; Ürük and Külünkoğlu İslamoğlu, 2019). The Breeam evaluation system uses accepted performance measures that adjust a building's features and design. The metrics used represent a broad range of criteria, from energy to ecology. These categories; It consists of building management, indoor health and well-being, energy, water, transportation, materials, waste, land use and ecology and environmental pollution (Saunders, 2008). The criteria that include these issues have a range of environmental weights depending on the countries where the buildings (structures to be certified) are located. Figure 4 shows the weights determined for Europe;

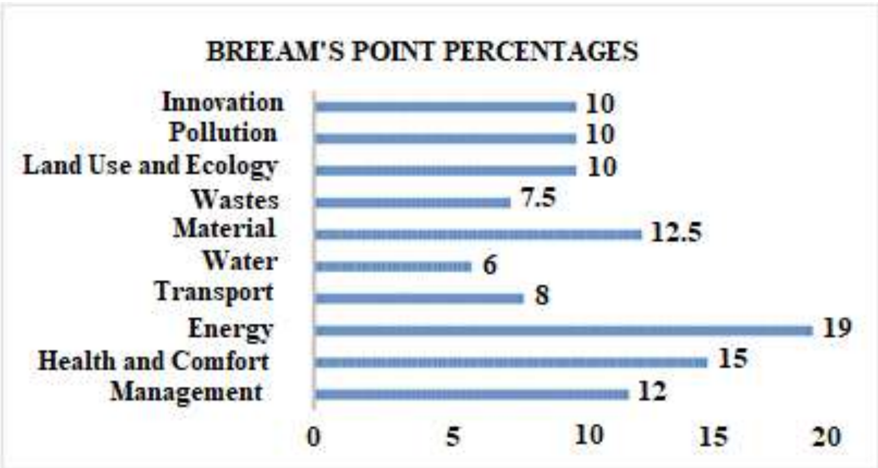


Figure 4. Percentages of the Breeam Certification System (Ürük and Külünkoğlu İslamoğlu, 2019).

Breeam has 5 different certification classes and is evaluated according to the points the building receives. The validity period of the certificate is planned to be three years. After three years have been completed, the building must be updated by applying to the certification system again. Different certification classifications are shown in Figure 5 below (Ürük and Külünkoğlu İslamoğlu, 2019).

Figure 5. Certificate Classes and Scores of Breeam Certification System.

b. Leed Green Building Certification Systems

The Leed Certification system was created in 1998 by the American Green Building Council (USGBC). It is an internationally accepted building certification system. The purpose of the certification system; To ensure that all individuals and organizations working in the construction sector draw their attention to environmental values and make decisions aimed at protecting the natural environment.

Leed is a points-based system and each building project earns Leed points for meeting certain green building criteria. In each of the seven LEED credit categories, projects must specifically meet certain prerequisites and earn points.

LEED certification; It can be used for all building types, including existing buildings, commercial interiors, schools and homes, new buildings under construction and renovated buildings undergoing major renovations. Leed Green Building Certification System has categories according to the type of use and location of the building, as stated in Table 6 (Ürük and Külünkoğlu İslamoğlu, 2019).

Figure 6. Categories of LEED Green Building Certification System

In order to obtain the LEED green building certificate, the building must gain a minimum of 32 points required to meet all the prerequisites in the subheadings of the main headings specified in Figure 7. Figure 7 shows the levels of the Leed certification system (Ürük and Külünkoğlu İslamoğlu, 2019).

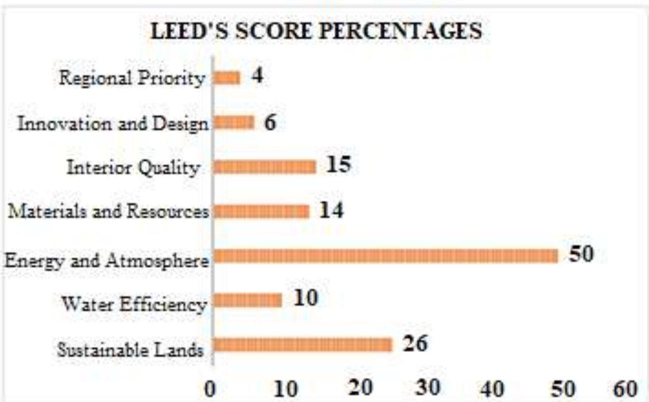


Figure 7. Score Percentages of the Leed Certification System.

The LEED certification evaluation process begins with the project's registration with the American Green Building Council (USGBC). After the documents are completed and sent, the final evaluation is made and the certificate level is determined and the result is notified to the applicant official. In this section, the project owner can accept the evaluation result or use his right to object. After objections, the building is certified according to these results. Leed has 4 different certificate classes (Gold, Platinum, Silver and Leed Certificate) and the building is evaluated according to the points it receives and there is no limit on the validity period of the certificate. Different certification classifications are shown in Figure 8 below (Ürük and Külünkoğlu İslamoğlu, 2019).

Figure 8. Certificate Classes and Scores of the Leed Certification System.

c. DGNB Green Building Certification Systems

DGNB (German Sustainable Building Council) was established in Germany in 2008 together with the Joint Ministry of Transport, Construction and Urban Affairs. It is intended to be used in the planning and evaluation of buildings as green buildings. While designing, LEED and BREEAM were taken as basis (Ürük and Külünkoğlu İslamoğlu, 2019).

The primary goal of the German Sustainable Building Council is the establishment and subsequent development of its own certification system. In this context, the DGNB Certification System was established for office and administrative buildings in 2009. This system was developed and became an international system in 2010, covering existing and new buildings, educational institutions and commercial buildings (Dgnb, 2011).

Figure 9. DGNB overall building assessment criteria

DGNB green building certification system has main objectives such as evaluating the use of energy resources, reducing the amount of energy consumed and using sustainable systems. Score percentages are shown in Figure 10.

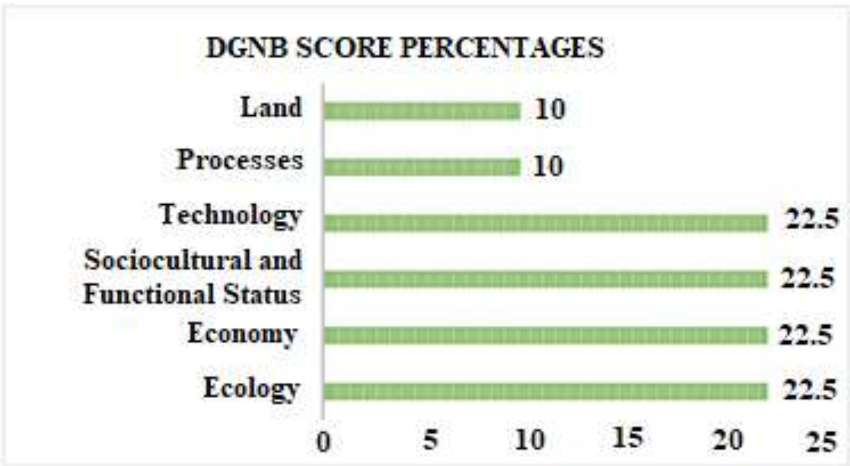


Figure 10. Score Percentages of DGNB Certification System

Each area is designed with different weights in accordance with the profile of the occupants and contains special criteria that can be monitored throughout the entire life cycle of the building. If the building meets the criteria, a DGNB certificate is received in the form of gold, silver or bronze, depending on the degree of fulfillment of the criteria (Figure 11). There is no limitation on the validity period of the certificate (Anbarcı et al., 2012; Dgnb, 2011).

Figure 11. Certificate Classes and Scores of the DGNB Certification System

DISCUSSION AND CONCLUSION

The most important factors of green building projects are their effects on human health and urban fabric. People should be able to live their lives in a healthy environment. In order to prevent the deterioration of human health caused by buildings, it must be ensured that the buildings are healthy. Studies on the sustainability of healthy buildings should be integrated by considering building, environment and human health together, they should be designed in a way to leave healthy living spaces to the next generation, and all segments of the society should be made aware of this issue.

With the increase in sustainability awareness, the issue of green building production has gained importance in the construction sector (Arslan, 2015). Green building projects are practices that have gained importance by taking into account the damage that buildings cause to nature and the environment on a global scale, and subsequently become increasingly widespread within the framework of certain standards and rules with the research and studies carried out on this subject. These standards and rules determine the sustainability criteria of green buildings; It explains how the green building development process should be qualitatively and enables quantitative measurement and evaluation of the building.

In countries that support green building practices, real estate development projects such as shopping malls, offices, hotels, housing projects and industrial facilities should be developed as green buildings. In addition to its environmentally friendly features, it is also important in terms of gaining prestige and increasing the value of the project and the developer company (Şenol, 2009).

The aim of green building design is to produce energy efficient living spaces by minimizing the consumption of natural resources. For this reason, passive and active systems should be used together in air conditioning and lighting, which are the systems that consume the most energy in buildings. To obtain a sustainable building, natural data should first be examined and passive approaches should be taken into account to provide maximum energy from natural resources, and then they should be supported with active systems to meet the targeted certification system requirements.

Each country differs from each other in terms of their climatic characteristics, geographical location, energy consumption and cultural structures. For green building certification systems to be effective and applicable, evaluation criteria must take into account national conditions, both in terms of climate and natural resource capacity, as well as economic and social conditions. For this reason, it is not correct to directly apply certification systems in other countries. Certification systems should be prepared taking into account the local conditions of each country (Arslan, 2015).

It is important to increase investments in green building practices for a sustainable future. In order for green building projects to become widespread, investors should be encouraged and the state and local governments should take initiatives for this. Obtaining a green building certificate should be made mandatory by local governments in new projects and especially in urban transformation projects, which are on the agenda today. In this way, the renewal of newly developing urban transformation areas with a sustainable approach is ensured. At the same time, a more sustainable environment will be offered to future generations thanks to increased investments in green building practices and widespread awareness of sustainable design (URL 6).

As a result, green buildings encompass a holistic approach to sustainability that includes a variety of features and practices to minimize their environmental footprint. By prioritizing energy efficiency, renewable energy sources, water conservation, responsible material selection and other key elements, green buildings contribute to a more sustainable and resilient building environment. Adopting green building principles reflects a commitment to environmental protection and a desire to create healthier, more efficient spaces for users.

REFERENCES

Anbarcı, M., Ö. Giran, İ. H. Demir. 2012. Uluslararası Yeşil Bina Sertifika Sistemleri İle Türkiye'deki Bina Enerji Verimliliği Uygulaması. e-Journal of New World Sciences Academy, 7(1): 368-383.

Arslan, N. C. 2015. Yeşil Bina Projelerinde Tasarım Süreci İçin Bir Yaklaşım: Leed V4 Sertifikalandırma Süreci Modeli. İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, İstanbul.

Benedict, M. A., E. T. McMahon. 2001. Green Infrastructure: Smart Conservation for the 21st Century, Sprawl Watch Clearinghouse, Washington.

Breeam. 2011. Breeam New Construction Non-Domestic Buildings Technical Manual SD5073-2.0.

Burnett, J. 2007. City Building, Eco Labels and Shades of Green, Landscape and Urban Planning, 83(1): 29-38, 12 November 2007.

Dgnb. 2011. Excellence Defined, Sustainable Building with a Systems Approach. http://issuu.com/manufaktur/docs/dgnb_excellence_defined_sustainable_building_with_/4?mode=a_p, 10-13.

Doğan, M., D. Seçme. 2018. Çevre Dostu Binalar ve Yeşil Bina Sertifika Sistemleri. Akademia Disiplinlerarası Bilimsel Araştırmalar Dergisi, 4(1): 19-27.

Erten, D. 2011. Yeşil Binalar, Sürdürülebilir üretim ve tüketim yayınları-V, Çevre ve Şehircilik Bakanlığı, Ankara.

Geçer, E., İ. Şentürk, H. Büyükgüngör. 2019. Yeşil Bina Tasarımında Su ve Enerji Yönetimi Üzerine Uygulama Örneği. GUFBED, 9(2): 332-343.

Gür, N. V. 2007. Mimaride Sürdürülebilirlik Kapsamında Değişken Yapı Kabukları İçin Bir Tasarım Destek Sistemi. Doktora Tezi, İstanbul Teknik Üniversitesi Fen Bilimleri Enstitüsü. İstanbul.

Hoşgör, H. 2014. Yeşil Hastane Konsepti ve Türkiye Deneyimi. Sağlık Yönetimi.

Kaylı, A. ve Güneş Gölbey, A. (2020). Yeşil Altyapı ve Yeşil Bina Bileşeni Olarak Kurakçıl Peyzaj Uygulamaları. Ege Üniversitesi Ziraat Fakültesi Dergisi, 57(2): 303-311, Doi: 10.20289/zfdergi.669799

Leed. 2011. LEED Certification for Neighborhood Development. http://www.usgbc.org/DisplayPage.aspx?CMSPageID=1988.

Saka, İ. 2011. Sürdürülebilirlik Açısından İstanbul'da Bir Ofis Binasının Leed Sertifikalandırma Sistemi Kapsamında Değerlendirilmesi. (Yüksek Lisans Tezi). İstanbul Teknik Üniversitesi, Mimarlık Anabilim Dalı, Ocak.

Saunders, T. 2008. A Discussion Document Comparing International Environmental Assessment Methods For Buildings.

Sev, A. 2009. Sürdürülebilir Mimarlık. Yem Yayın, İstanbul.

Sur, H. 2012. Çevre Dostu Yeşil Binalar. Yeşil Binalar Referans Rehberi, İstanbul.

Şenol, S. 2009. Gayrimenkul Geliştirme Sürecinde Yeşil Binaların Sürdürülebilirlik Kriterleri Açısından İncelenmesi. (Yüksek Lisans Tezi). İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, İstanbul.

URL 1. Yeşil Bina Nedir? Yeşil Bina Özellikleri Nelerdir? 2023-05-25, https://villayapi.com/tr/blog/yesil-bina-nedir/

URL 2. Sentrum. Yeşil Bina Nedir? Yeşil Binalar Hakkında Her Şey! https://www.sentrum.com.tr/blog/yesil-bina-nedir-yesil-binalar-hakkinda-her-sey

URL 3. Dünyadan Sürdürülebilir 5 Yeşil Bina Örneği. https://www.austrotherm.com.tr/bilgi-servisi/haberler/duenyadan-suerdueruelebilir-bes-yesil-bina-oernegi

URL 4. Yeşil Bina Özellikleri Nelerdir? https://erketasarim.com/blog/yesil-bina-ozellikleri-nelerdir

URL 5. Yeşil Bina Danışmanlığı. https://www.dilekasan.com/yesil-bina/

URL 6. Yeşil Bina Özellikleri Nelerdir? https://erketasarim.com/blog/yesil-bina-ozellikleri-nelerdir

Ürük, Z. F., A. K. Külünkoğlu İslamoğlu. 2019. Breeam, Leed ve DGNB Yeşil Bina Sertifikasyon Sistemlerinin Standart Bir Konutta Karşılaştırılması. Avrupa Bilim ve Teknoloji Dergisi, 15, 143-154, Doi: 10.31590/ejosat.512291

Yılmaz, M., S. Yıldız, Gültekin, A. B. 2016. Yıkıcı Yeniliklerin Belirlenmesi: Sürdürülebilir İnşaat Sektöründe Tehditler ve Fırsatlar. Balıkesir Üniversitesi Fen bilimleri Enstitüsü Dergisi, 18(2): 25-36.

Yücel Işıldar, G., A. Gökbayrak. 2018. Yeşil Binalarda Belgelendirme Ölçütlerinin Ülkelerin Gelişmişlik Düzeyine Göre Değerlendirilmesi. Ömer Halisdemir Üniversitesi Mühendislik Bilimleri Dergisi, 7(1): 46-57.

VIGOR OF VEGETABLE SEEDS – ESSENCE, HISTORICAL DEVELOPMENT, SIGNIFICANCE AND PERSPECTIVES

Nikolay Panayotov[1]

[1]*Agricultural University – Plovdiv, Bulgaria, 12 "Mendeleev" Str. 4000 Plovdiv*

Corresponding author e-mail:: nikpan@au-plovdiv.bg

ABSTRACT

Most vegetable crops are grown through seeds and this determines the need to establish their viable potential. The main element in this direction is the standard germination test, which is widely applied and is globally recognized for the quality of individual lots. Although germination is the backbone of seed quality, it does not provide sufficiently complete and real information about seed behavior under field conditions. This necessitates looking for other indicators for a more complete assessment of their vital status. Vigor adds to the characterization of the properties and potential of the seeds by noting, in addition to their ability to germinate, the ability of young sprouts to develop under a wide range of environmental conditions. Establishing vigor is particularly important in vegetable seeds which are small and most are hybrid and heterosis and with high price. This article is an overview of the development of the vigor concept historically, its advantages and challenges for agronomic science. The specifics of the individual tests for which there are already recognized protocols for their application and their importance based on own research on the quality of seeds from different species of vegetable crops are followed. Attention is also paid to the modern requirements for creating fast and non-invasive tests. Some newly developed vigor determination methods suitable for use with vegetable seeds are also indicated. The author's new methodology for determining vigor, "Initial Vegetative Seed Productivity", is described in details. The difficulties, the not completely solved problems, as well as the perspectives for the introduction of vigor as a standardized indicator widely adopted worldwide to evaluate the potential of individual lots of vegetable seeds are analyzed.

Keywords: seeds, germination, tests, germination, sowing quality, viability

INTRODUCTION

The beginning of our civilization began when people 10,000 years BC discovered the seed, realized its role and essence and began the development of organized agriculture. This marks the beginning of settled life and the construction of settlements (Daniels and Hysloop, 2003; Isbouts, 2009; Marcos-Filho, 2015). Already in ancient times, man perceived the unique nature of seeds, especially the phenomenon of germination, the weave it into various philosophical teachings and included them in many religious beliefs and rituals, since the time of the ancient Egyptians. This is also evidenced by the works of one of the first recognized botanists, such as Theophrastus (370-285 BC), who, in addition to describing the seed, emphasized its huge role in the existence of not only peoples but also in nature (Evenari, 1980).

With the growth and systematization of targeted studies in the field of seed science, Broniewski et. al. (1976) points out that the basic dialectic laws are manifested in seeds, and their appearance is the result of a long evolutionary process, which makes it extremely adaptable and thus seed-propagating plants occupy a leading role in nature. Research on seeds also provides information on most of the properties and characteristics inherent in plants, since in the seed itself, as a living organism, most of the processes that take place in adult plants are observed. That is why the seed can definitely be considered a peculiar microcosm (Copeland and Donald, 2001).

Of the world's cultivated plants, over 75-80% are propagated and produced by seeds, including the majority of vegetable species (Marcos-Filho, 2015; Welbaum, 2024). The growth of population, the increased urbanization, the economic development, and the changes in eating habits cause an increase in the consumption of vegetables. To meet these needs, a significant increase in production is necessary, which in turn requires the supply of sufficient and high-quality seeds. Because of this, and appreciating the great importance of seed quality, in the 19th century, seed production emerged as an independent and specific branch of agriculture, first in Europe and then in the USA (Kastler, 2005). The large number of types of vegetable crops leads to some fragmentation and careful organization and management of the processes from the production to the market of vegetable seeds is particularly necessary. Data on the world market of vegetable seeds and forecasts for its development point a continuous growth in their sales.

It is pointed out that the global vegetable seeds market in 2022 was approximately USD 8 billion, in 2023 it reached USD 10.92 billion, and it is predicted to exceed USD 12.15 billion in 2024. Forecasts predict that sales of vegetable crop seeds during the period 2028-2031 will increase significantly and reach $18.48 billion, but there are even more daring forecasts that indicate an even much larger amount. This market is very dynamic and highly competitive, as evidenced by the fact that about twelve global companies, eight of which specialize in the production of vegetable seeds, supply seeds for thirty crops and have annual sales of over $100 miloin. This enables between 15% and 30% of the revenue from these sales to be directed to the development and research of new technologies related not only to the production, but also to the evaluation and stabilization of the vital status of the seeds of vegetable crops. The majority, about 80% of the world sales of vegetable seeds is concentrated in ten countries and these are: China, India, Japan, South Korea, France, Italy, Spain, the US, Mexico and Brazil. In recent years, approximately 80-90% of vegetable crops have been produced from hybrid and heterosis seeds, the cost of which is much higher (Shoham, 2024; Report, 2024).

That is why the need for the full and real establishment of the quality and vital status of the seeds stands out in the foreground, to guarantee a stable and sustainable production of vegetables. The backbone for assessing seed viability potential is currently perceived to be primarily the determination of germination by the standard test. Indeed, this is one of the most important, but not sufficient indicators for a complex and complete characterization of the seeds of vegetable crops, and as stated above, in addition to the fact that their price is significantly higher, but also the demand and sales are constantly growing. Several authors point out that the globally accepted and standardized approach to seed quality, only employing of their germination, does not provide complete information about their possibilities and manifestations under field conditions, and therefore it is necessary to look for new approaches in this direction. Scientists in the scope of seed science agree that the best results can be obtained by introducing vigor as an element for the overall characterization of the sowing qualities and potential of seeds, including those of vegetable species. The essence of vigor is to compare and distinguish stronger from weaker lots of seeds and it is appropriate to apply to lots that have high germination (Copeland and McDonald, 2001; Black et al, 2008; Panayotov 2016).

This also determined the direction of this review. Its main aim was to trace the development of the concept of vigor in historical terms, its advantages, weaknesses and challenges for agronomic science, to highlight its great importance for evaluating the seed potential and specificity of its application in vegetable seeds, as well as to outline the prospects in this area. This review has no pretensions to be comprehensive of the abundance and diversity of aspects of the ever-increasing research and emerging trends in the field of vigor, which is impossible.

VIGOR CONCEPT

The potential of the seeds is mainly determined by the indicators of vitality, viability and ability to develop normal seedlings, i.e. the level of vigor. They are important both for scientific research and directly in agronomic practice. To understand the specifics of individual analysis and delineate the meaning of vigor, it is appropriate to briefly indicate the differences in the mentioned terms (Dencheva et al., 1985).

- *Vitality* are those seeds that have a living embryo, determined by various methods;
- Due to the fact that not all seeds that are viable can germinate, due to many different reasons, the term *viability* is introduced, and these are normally germinated seeds under certain laboratory conditions;
- Of course, under production and field conditions, these seeds will not show the same vitality, nor can they be evaluated by this indicator for the possibility of developing plants and forming enough biomass. All this necessitates the search and application of new methods for evaluating the so-called seed *energy* or *force*.

Depending on the point of view and the direction of using the seeds, there are different concepts about the essence of vitality. In most cases, seed producers and sellers perceive the seed as an object that may or may not germinate. However, a deeper insight into the nature of seeds reveals that the seed should be considered as a living organism in which metabolic processes take place, there is enzymatic activity, there is the presence of living and dead tissues and this determines its ability to germinate.

A number of publications emphasize that under the standard method of germination, germination is limited only by the ability of the seed to develop the embryo, i.e. those essential structures for the various types of seeds that determine the possibility of its reproduction, but under favorable conditions, without determining the developmental potential of this sprout and subsequently the young plant under uncontrolled field conditions (AOSA, 1991; Copeland and McDonald, 2001). Although this standard test has good repeatability and can be applied anywhere, it also has its weaknesses.

The main disadvantages of germination by standard test are in several aspects: i) substantial overestimation of seed properties, since the standard test is conducted under controlled, artificial near-sterile conditions that are never encountered in the field; ii) germination is the sum of germination of stronger and weaker seeds, respectively counted at an earlier and at a much later stage, which is a big disadvantage, because under field conditions the weaker seeds would not be developed in this way; iii) there is also a lack of a precise scale for reporting and for distinguishing individual lots as capable of developing weak and strong seedlings, as well as the associated subsequent, expected and natural deterioration of the seeds; iv) does not state the rate, "energy" and "force" for growth, which are essential elements for normal plant development thereafter (McDonald, 1993; Panayotov, 2016).

One of the main postulates in vigor concepts is based on the changes in various processes and properties of the seed leading to its earlier deterioration compared to germination. Delouche and Boskin (1973) described a hypothetical model for the sequence of possible changes causing a reduction in seed potential (Figure 1). According to them the vigor is much more sensitive and any change before germination loss occurs can serve as a basis for its determination. According to this model, degradation of cell membranes occurs first before the decrease in viability is observed. This disruption of the integrity of the cell membranes creates the conditions and determines to a significant extent the greater sensitivity of vigor. It gradually reduces energy, biosynthetic processes and respiration, and only then is the deterioration of germination noticeable. A decrease of the storability, resistance to pathogens and development of abnormal seedlings is reached, and a loss of germination is also reached. This shows that even before the loss of germination, the changes occur in a number of processes that are indicators of changes in vigor values. This is how the individual approaches to the assessment of vigor can be outlined.

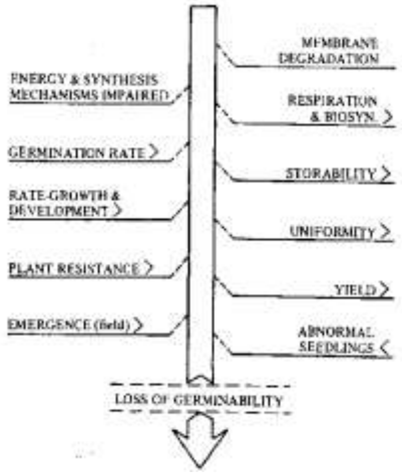


Figure 1. A putative sequence of changes occurring for seed deterioration (by Delouche and Baskin, according Copeland and Mc Donald (2001)

When considering the concept of vigor, it is imperative to note that there is a very strong relationship between vigor and seed germination (Delouche and Caldwell, 1960). The stated opinion about earlier changes in vigor before germination is an excellent prerequisite for earlier prediction of the changes that occur in the life potential of the seeds. In this way, the determination of vigor has points of contact with their storability. Several authors, although in some cases hypothetical, note that significant changes during storage in vigor can be observed long before they have occurred at such a level in germination. Justice and Bass (1978) and Dentcheva (1985) divided the process of changes of these two features into three periods (Figure 2). From the graph, it is clear that during the first stage, vigor loses 25% of its initial value, then germination is 90% and above 90%. In the second, the losses of vigor increase to reach a complete loss in the third period. That's why if it's used only the standard germination test these seeds would be class one. The changes in the curves are often sigmoidal but maybe and approach a weakly linear appearance.

It is analogous to the conclusion of Marcos-Fliho (2015) that initially, the loss of vigor tends to a parallel loss of vitality, then vigor decreases very rapidly and finally, the rate of loss slows, but zero values of vigor are reached and death of all occurs seeds (Figure 3).

In support of this opinion are the conclusions made by Panayotov (2023) when studying the life potential of cape gooseberry seeds during their natural aging. It has been established that, in addition to germination, determination of vigor is of much greater importance for determining seed quality. Comparing the data obtained on germination and vigor after 7 years of storage of harvested seeds, the conclusion is formulated that, compared to the initial values, the decrease in vigor is much faster than that of germination, and this is observed very clearly after the third year of storage, when vigor has decreased by 20-25%, and the germination decreased by about 8-9%. While in the sixth year, vigor loses about 32-35% and germination only 10% (Figure 4).

Similar are the findings of another experiment (Panayotov 2010a), conducted at the Department of Horticulture of the Agricultural University-Plovdiv, Bulgaria with seeds of two typical Bulgarian pepper varieties aged under laboratory conditions. When comparing the changes in germination and vigor, relative to the values for the first year, taken as 100%, it is seen that vigor decreases much faster than germination. In one variety, when germination decreased by 20%, vigor decreased by 58.2%.

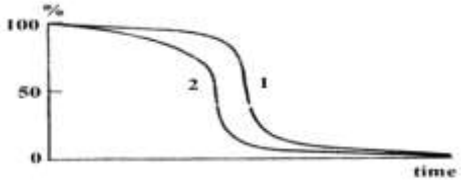


Figure 2. Changes of viability and vigour of the seeds during the period of their storage (by Justice and Bass, according Dencheva, 1985)
1-dynamic of the changes of viability;
2 – dynamic of the changes of vigour

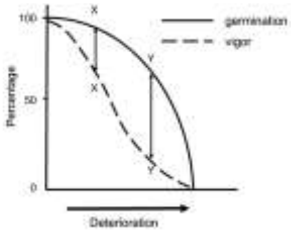


Figure 3. Relationships between seed germination and vigor in association to the progress of deterioration (by Marco-Fliho, 2015)

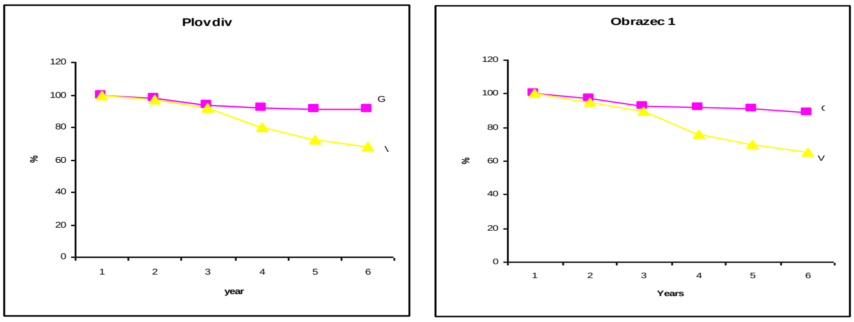


Figure 4. Changes of the germination (G) and vigor (V) after seventh year of storage of cape gooseberry seeds (by Panayotov, 2023)

From the above, the need to search for new ways and methods for full monitoring of the physiological potential of seeds during the various stages of their production, storage, trade and application is outlined. It is necessary to establish the potential not only for the germination of the seed but also for its ability to develop into a plant, due to the presence in it of the above-mentioned "power" or "energy"

HISTORICAL DEVELOPMENT OF THE DOCTRINE OF SEED VIGOR

The historical development of the scientific foundations, theory and practice of seed vigor is very well described by McDonald (1993) and Marcos-Filho (2015). The concept of the presence of such "power" and "energy" in seeds arose as early as the second half of the 19th century.

Much earlier, in 1816, the first seed law was published in the city of Bern, Switzerland, with a view to the regulation of trade, which aimed to introduce the basic conditions and standards to which seeds in trade must meet. The reason for issuing such a law is interesting, it is because unscrupulous traders used to put small pebbles in the packages of clover seeds.

The pioneer in laying the foundations of the doctrine of vitality and especially the vigor of seeds was Frederick Nobbe. In 1869, in En Tharandt, near Dresden, Germany, he established the world's first seed testing institution, the Seed Testing Laboratory, under the name

"Physiological Research and Seed Control Station in Tharandt". This positive example was soon followed in other countries. In 1876, E. H. Jenkins established the first such laboratory in the United States in the state of Connecticut. A decade later, in 1884, such a laboratory was also organized in France, the Station National d'Essais de Semences (Kastler, 2005).

A few years later, in 1876, Nobbe for the first time in his scientific work "Handbuch der Samenkunde" evaluated the physiological potential of seeds and expressed the opinion that they contained the so-called "growth power". In 1911, his student Lorenz Hiltner together with another researcher Ihssen, introduced the term "triebkraft" which means "driving force" and "launch force" or "pushing force" of seedlings. In this way, they denote the "stronger" seeds that form larger roots compared to the "weaker" ones of the same lot that form shorter roots. These two researchers also created one of the first tests to assess vigor called the "brick grit test" (Marcos-Fliho, 2015).

The interest in assessing the quality of seeds and their potential increased and in 1905 in France issued the first seed quality control law, followed by the USA in 1917, where in the state of Connecticut, the first unified and standardized rules for qualification of the seeds and sowing material were published. ("Rules for Testing Seeds") (McDonald, 1993; Heaton, 2015; Panayotov, 2016). Some of the first terms, in this relatively new field of science, to describe the concept of seed vitality included "driving force" and "germination", as well as seedling 'vigor' or energy of seedlings (McDonald, 1993).

According to Marcos-Filho (2015), in this early period, around the 30s of the 20th century, research in this area was also related to the development of other methods and tests for assessing the physiological potential and vigor of seeds, such as the Electrical Conductivity Test from Fick and Hibbard (1925), estimation of germination rate by Stahl (1931), and the first observations of the cold test (Alberts, 1927; Tatum and Zuber, 1943).

After the 1930s, although there were active discussions in the USA to introduce the term "germination energy" to express the speed of this process, the attention of scientists about the potential of seeds and the development of evaluation methods decreased. In this period, as mentioned by França-Neto et al. (1998), in 1940 Lakon introduced the tetrazolium test (Marcos-Filho, 2015).

In the middle of the 20th century, the interest of scientists in the field of vigor was revived again. This happened in 1950 at the Congress of the International Seed Testing Association (ISTA) held in Washington. During this meeting, the President of ISTA, W. J. Frank pointed out that the international trade in seeds, after the Second World War, was growing, but more and more difficulties were encountered due to the discrepancy between the results of the tests carried out in Europe and the USA. According to him, this arises from the different understanding and philosophy of the standard germination test. The differences are expressed in the fact that in Europe the reproducibility of the standard germination test is of greatest importance and this is the basis for international trade, while for America the essential is the ability of the seeds from individual lots to form a normal plant. He recommends smoothing over these differences in philosophy. At the same time, offers a specification of the terminology for establishing the potential of the seeds, distinguishing two directions. One is for the standard germination test, which is mainly prevalent in Europe, to be carried out under artificial and optimal environmental conditions to have repeatable results. The other one is for the test to evaluate the percentage of developed seedlings, conducted under significantly closer to field conditions, a methodology developed more in the USA and a term for its name is proposed. Thus, a new term "Vigour" or "Vigour Test" was adopted to evaluate the physiological potential of seeds under suboptimal and close to real conditions (Franck, 1950). In that year, Franck founded the ISTA Biochemical and Seedling Vigor Committee with two main goals: to define the concept of vigor and to develop a standardized test for its determination.

From that moment, a change in scientific research began, with more emphasis now on methods for determining vigor, to establish the effect of conditions influencing a more complete assessment of the seed's capabilities. Thus began a gradual increase in the importance of vigor as a routine test, especially for grain and vegetable crops. However, as McDonald (1993) points out, it is widely believed that this is primarily a matter of resolution in Europe, where the standard germination test is of primary importance.

A significant boost in scientific research was observed in the 1960s, deepening studies on seed physiology to incorporate them into more complete tracking of seed performance. Efforts to develop universally accepted definitions and concepts of vigor are beginning to be more active. Methods for several tests such as the tetrazolium test, electrical conductivity and accelerated aging are being improved. According to Woodstock (1973) and Marcos-Filho (2015), the number of scientists starting to work in the field of seed science and in particular with a direction towards vigor is increasing. Seed producers and agronomic scientists began to pay more and more attention to the importance of vigor, and this led to the systematization of information and the development of the necessary documentation. Scientific studies are deepening to reveal the mechanisms of seed aging leading to reduced vigor. Emphasis is placed on the importance of such basic processes that begin to deteriorate the vital status of seeds and, accordingly, vigor, such as the degradation of cell membranes, lipid peroxidation, changes in the state of the cytoplasm (Priestley, 1986; Wilson and McDonald, 1986; McDonald, 1999). Studies in this scope are becoming more and more diverse. Scientific fields are expanding the beginning of research on the processes leading to changes in vigor. Thus, vigor becomes more and more a comprehensive indicator, and for its determination, knowledge from a number of sciences is included, and thus the study acquires an increasingly interdisciplinary character, providing an opportunity to reveal much more real information about the essence and character of the power and potential of seeds.

The development of the doctrine of vigor was also inextricably linked to the work of the AOSA Vigor Test Committee established in 1961, the main purpose of which was to indicate the advantages and disadvantages of direct versus indirect vigor tests and to formulate the concepts of vigor testing. It began to develop actively in 1968, but there are still two opinions, according to one the tetrazolium test is the most suitable, i.e., an indirect assessment, and according to others, direct measurements are the most important. In 1974, the idea of standardizing vigor tests arose. In 1983, the Committee already published the main tests that are appropriate to apply and these are: the accelerated aging test, cold test, cool germination test, conductivity test, seedling vigor classification, seedling growth rate and tetrazolium test. During the period 1987-1991, there was active work on the standardization of these tests, the first officially proposed test being for accelerated aging of seeds. The number of laboratories that apply them is also increasing, from 52% in 1978 to 75% in 1990 (McDonlads, 1993).

DEFINITION OF VIGOR

In parallel, the need to create a precise and scientific definition of vigor is growing, because without her presence, the measuring an ill-defined object is difficult, even impossible (McDonald, 1993). Many definitions are offered, but at the beginning, an opinion arises that already accepted, existing seed vigor is so comprehensive that it is very difficult to formulate a correct and precise concept, which is practically not far from the truth. The first attempt at a meaningful definition was made in 1957 by the American botany professor Duane lsely, who proposed that vigor should be understood as "the sum total of all seed attributes which favor establishment under favorable conditions" (lsely, 1957).

A few years later, in 1960, J.C. Delouche (Agronomist in Charge, Seed Technology Laboratory, Mississippi State University, Mississippi State, USA), and W.P. Caldwell (Seed

Technology Laboratory, Mississippi State University, Mississippi State, USA) in an attempt to upgrade this definition, express the opinion that "seed vigor is the sum of all seed attributes which favor rapid and uniform stand establishment", i.e. for germination and creation and development of a plant. Thus, a significant step is taken for a correct and accurate definition of vigor, because on closer inspection it is seen that there is a significant difference between the two definitions. In the definition proposed by J.C. Delouche and W.P. Caldwell foregrounds the stand establishment to emphasize rapid and even development and remove the need for "favorable conditions" present in Duane lsely's definition. Thus, one main point is clarified, namely that the rapid and uniform development of seedlings under field conditions is an extremely important characteristic of seed vigor (McDonald, 1993).

However, the question arises regarding the "sum total of all seed attributes", what are these factors and conditions that determine seed vigor. For greater clarity in the definition, Woodstock (Market Quality Research Division, USDA, Beltsville, Maryland) in 1965 proposed that vigor is "that condition of good health and natural robustness in seed which, upon planting, permits germination to proceed rapidly and to completion under a wide range of environmental conditions." In this definition, the most important point is the wide range of environmental conditions. Pollock and Roos (1972) accept that "seed vigor might be considered as a potential for seedling establishment in the field". Then this idea was widely adopted by seed producers.

A year later, in 1973, Perry supplemented the definition, understanding vigor "physiological property determined by the genotype and modified by the environment which governs the ability of a seed to produce a seedling rapidly in soil and the extent to which the seed tolerates a range of environmental factors." Thus, it is already clearly pointed out that vigor depends on both genetic prerequisites and environmental conditions (McDonald, 1993).

In 1977, ISTA created an official definition of vigor "the sum of all those properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence". Three years later, in 1980, AOSA refined this definition and it became "...those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions." A positive feature of this definition is that parameters are used that are numerically measurable, such as rapid and uniform germination, as well as the well-known "good development of seedlings". It is also important to emphasize the ability of seeds not only to develop in the field but to do so under a wide range of environmental conditions, i.e. under both optimal and stressful conditions. In this way, the essence of vigor is very clearly distinguished from that of seed germination. It should be emphasized that it is appropriate to subject lots to the vigor test which has already shown good germination in the standard test.

Marcos-Filho (2015) also points out some weaknesses in the understanding of vigor, arguing that the available concepts do not cover the development of plants throughout their life cycle up to yield and that the index by itself does not represent a distinct physiological process. He emphasizes, however, that the emergence of vigorous seedlings, due to their uniform and accelerated development, can lead to an increase in productivity, and seeds with high vigor germinate much more evenly and have better stability. According to him, vigor is manifested as a result of the interaction of many physiologically independent characteristics of the physiological potential of seeds, such as: germination rate, seedling growth, ability to germinate above or below optimal temperatures and other aspects of stress tolerance. This, in turn, makes it difficult to formulate a precise definition, since many and varied factors are involved in the manifestation of vigor, and thus it is much easier to understand the main effects of vigor than to define it. Panayotov (2015) also reported that vigor depends on many factors: genetic features, trauma to the seeds, the chemical composition of the seeds, environmental conditions during development, ripening and harvesting as well as maturity and storage.

VIGOR TEST

According to Marcos-Fliho (2015), vigor tests should be much more sensitive to changes that cannot be detected by the standard germination test. The purpose of the vigor test is to determine the differences in the physiological potential of seeds from different batches with similar germination and their ability for better development of seedlings under field conditions, as well as for higher storability. Vigor cannot indicate the number of plants developed in the field or the storage period, but the correct interpretation of the results allows to select the best batch of seeds and to specify the sowing rate and the appropriate method of sowing. Direct or indirect methods are used to determine the metabolic activity, the physiological state of the seeds and the tolerance to stress conditions. The first test that was officially included in the International Rules for Seed Testing of ISTA in 2001 is the electrical conductivity test, applied to garden pea, bean and soybean seeds. Currently, these rules also recommend the accelerated aging test for soybeans, the controlled deterioration test for *Brassica* spp. and the emergence of primary roots for corn. The need to expand the scientific activity in the field of vigor in the seeds of vegetable crops is also indicated by the fact that in an analysis of the systematized literature on the vitality potential of seeds out of 1158 references, only six were on the subject of seed vigor, and most of them are for field crops and very little for vegetable crops. A number of authors (Panobianco and Marcos-Filho, 2001; Bennett, 2002; Sako et al., 2002, by Contreras and Barros, 2005) support this opinion and point out that the results obtained for vigor from some tests in vegetable crops are insufficient and contradictory and strongly highlight the need to deepen the studies on vigor in the seeds from vegetable crops.

Many scientists, such as McDonald (1999), Copeland and Mc Donald (2001) and McDonald (2001) point out that the creation of a vigor assessment test should be related to the determination of one or more quantifiable parameters related to deterioration in vigor. The results obtained apply to the whole lot, but a lot consists of many seeds, each of which has its own plant development abilities. Tests are an analytical procedure for establishing the potential of seeds and must be standardized to compare data between individual lots. Vigor tests should be applied to batches with proven high seed viability and germination.

Types of vigor tests

The basis of the creation of a vigor test is the measurement of various parameters, one or more morphological, physiological, biochemical or other characteristics. According to Copeland and McDonald (2001), depending on the method of measurement, they are divided into direct or indirect.

Direct tests are carried out under conditions close to the real ones in the field, investigating the possibility of germination and resistance to stressful conditions. A disadvantage of these tests is that the difference in the status of the seeds from individual lots is not manifested when the seeds are placed under favorable conditions. Such is the test of low temperatures.

Indirect tests are most often based on changes in physiological processes in seeds. An electrical conductivity test can be specified in this group. However, a negative feature is that they cannot fully determine the physical and physiological conditions responsible for germination.

According to the indicators that are included for measurement, the tests can also be *physiological, biochemical and physical*. The *physiological* ones measure the germination and development of seedlings, the *biochemical* ones - the changes in chemical reactions such as respiration and enzyme activity, responsible for germination and here belong the electric conductivity and tetrazolium test, while the *physical* ones mainly cover parameters of size, density, texture or other factors of this character related to germination.

The tests can also be grouped as stressful and quick. *Stress tests* include the accelerated aging test, cold test, the test of low temperature, the test of cooling, osmotic stress, and aspects of germination and seedling morphology. Rapid tests admittedly require less time, they are primarily based on some chemical and physical processes. This includes the tetrazolium test, test of conductivity and enzymatic activity tests. There is another group - tests for germination and seedling development, and these are determination of germination energy (first count of germination), normal developed seedlings and imagine analysis (Bennett, 2002; Contreras and Barros, 2005).

Vigor test requirements

Vigor tests have specific requirements for them to be applicable and subject to unification and standardization (Copeland. and Donald, 2001; Contreras and Barros, 2005; Panayotov, 2016). That, they're like that:

- Be more *sensitive* than the standard germination test
- To be *cheap* - to require minimal costs, equipment, laboratories and labor;
- To be *fast* - to provide quick information, to avoid the peak workload of the laboratories, for the analyses to require little time and space for their implementation;
- To be performed *easily* - i.e. no special training of personnel, complex equipment and the procedures themselves should be simplified;
- To be *objective* - the parameters should be expressed numerically and measured quantitatively, which will facilitate the interpretation of the data and will enable the classification of the individual batches the seed performances;
- To be *repeatable* - an extremely important requirement, the results should be similar, if not the same, in the next execution and thus obtain comparability between individual laboratories;
- To have a dependence on *the real conditions* of the field. Many of the tests pay serious attention to this requirement to predict the behavior of the seed under field conditions.

Kinds of Vigor Assessment Tests

The specific diversity of vegetable seeds requires a very good knowledge of the particularities of the individual tests, their positive and negative sides and the possibilities of application depending on the species of the given crop. In this review I focus only on some of the more widely used and more important vigor tests that are more suitable for evaluating vegetable seeds.

Accelerate aging test

This test is one of the most widely used and is relatively well-researched and developed. It is officially recognized and included in the International Rules for Seed Testing of ISTA (2019), as a soybean seed vigor test. It covers many of the characteristics that a vigor assessment test should meet.

As early as 1915, Crocker and Groves (1915) suggested that protein coagulation occurs during seed storage and that this is due to an increase in temperature in the seed mass. Initially, this test was applied to assess the storability of the seeds (Helmer et al., 1962) (by Marcos-Fliho, 2015). The test is based on the notion that when seeds are hydrated at high humidity and temperature, their life potential decreases. Its essence consists precisely of processing the seeds under such conditions, with an average temperature of 41^{o}C and a relative humidity of about

100%. Higher temperatures are not suitable, because instead of creating stressful conditions, it is possible to reach denaturation of the protein and, accordingly, complete death of the seeds. Increasing the temperature above 43^{0}C or 45^{0}C will cause metabolic activity to cease, especially in low-vigor batches (Marcos-Fliho, 2015).

The application of the test is carried out in a specially designed plastic box with precisely defined dimensions (11 x11x 3 cm) and volume (363 cm^3) with a grid for placing the seeds, and 40 ml of water is poured into the bottom. In addition to water, in some cases, especially for smaller seeds, a salt solution can be used - 40 g NaCl in 100 ml of water (Jianhua and McDonald, 1996), thus reducing the absorption of water for the deterioration of seed quality and the development of microorganisms. It is assumed that this moderate increase in seed water content at high temperatures creates conditions closer to those of natural aging compared to the presence of only water at the bottom of the box.

The box is placed in a thermostat with the appropriate temperature. After the specified treatment period, the seeds are subjected to the standard germination test. Batches with higher vigor overcome this aging better and show higher germination (Baalbaki et al., 2009). The application of this test is directly related to the initial moisture content of the seeds.

The test was considered promising and in 1973 Delouche and Baskin improved the methodology. The initial variant of the officially adopted protocol was developed by McDonald and Phannendranath (1978). Marcos-Filho (2015) reports that this method, especially with the use of salt solution, is actively developing tests in many vegetable crops such as pepper, broccoli, carrot, cauliflower, cucumber, eggplant, lettuce, melon, onion, pumpkin, radish, spinach, tomato. Piana et al. (1995) found that accelerated aging of onion seeds for 48 hours at a temperature of 42^0 C are suitable conditions for vigor assessment. To evaluate the vigor of onion seeds, Rodo and Marcos-Filho (2003) indicate that the saturated salt accelerated aging test for 72 hours, at a temperature of 41^{0}C, is a very promising regime.

Panayotov (2014) studied accelerated aging in three batches of pepper seeds at 35^{0}C, 40^{0}C and 45^{0}C for 24, 48 and 72 hours. It was also found that at 45^{0}C, it caused almost complete seed lethality, making this temperature unsuitable for assessing pepper seed vigor. He pointed out that the appropriate regime for accelerated aging and determination of vigor of pepper seeds, where individual lots exhibited the highest physiological sensitivity, was 40^{0}C for 48 hours (Figure 5).

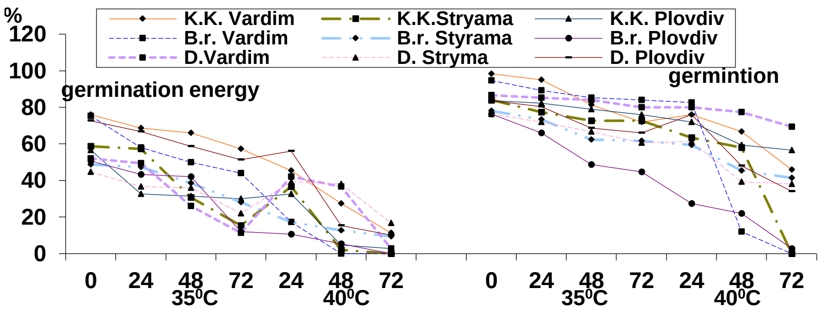


Figure 5. Accelerate ageing regimes for estimation of pepper seed vigor (Panayotov, 2014)
K.K.-Kurtovska kapia 1619. B.r. Bulgarski rotun, D-Delikates

The application of accelerated aging according to Svetleva and Panayotov (2014) is also a suitable method for assessing the viability of bean mutants. They found that the determination of vigor in these seeds is suitable to be carried out also at a temperature of 40^{0}C for 48 hours when high physiological sensitivity and well-defined differences between different seeds are observed (Table 1).

Despite an increase in scientific research on the use of accelerated aging in vegetable seeds, no sufficiently complete and definitive results have yet been formulated to establish a standardized protocol for such a test. It is similar to the opinion of Powell (1995) that the results of accelerated aging of vegetable seeds and small-seeded species are not completely unidirectional, since these small seeds absorb water much faster, their content varies greatly and this leads to a lack of uniformity between the individual lots (Marcos-Fliho, 2015).

Table 1. Sowing qualities of bean seeds after accelerate ageing (Svetleva and Panayotov, 2014, by Panayotov 2016))

Variants / Mutant	Germination energy (%)			Germination (%)		
	1	2	3	1	2	3
Control	61.6	80.0	71.6	84.3	88.3	86.7
24 h /40^0C	70.0	79.6	71.6	83.3	84.6	85.7
24 h /45^0C	45.0	45.0	48.3	46.66	55.0	66.6
24 h /50^0C	18.3	21.6	41.6	30.6	33.3	61.66
48 h /40^0C	61.6	78.3	65.0	72.6	81.6	74.0
48 h /45^0C	3.33	45.0	28.3	3.33	48.3	35.0
48 h /50^0C	1.66	11.6	21.6	1.66	18.3	28.3
72 h /40^0C	8.33	30.0	45.0	8.33	40.0	45.0
72 h /45^0C	5.0	13.3	13.3	6.7	13.3	16.6
72 h /50^0C	1.66	3.33	3.3	1.66	5.0	5.0

1 – Mutant with 0.0031 M NEC, 2; 2- Mutant with 0.0125 M EMS; 3- Mutant 0.0125 M EMS

The seeds of vegetable crops are characterized by different weight of 1000 seeds, and the porosity of the seed coat, which also determines the different absorptive capacity. This requires that the exact amount of seeds placed in the box be precisely specified. Furthermore, the appropriate regimes for accelerated seed aging of a lot of the major vegetable crop species, both in terms of temperature and duration of treatment, have not been firmly established. All this determines the need for additional and in-depth research to develop and standardize tests for accelerated aging in vegetable species. Advantages of this test is that it is sensitive, not expensive, easy for application, objective and repeatable. A disadvantage of this test is that it is not one of the rapid ones, due to the time required for processing the seeds and subsequent testing by the method of the standard germination test.

Electrical conductivity test (Conductivity test)

It was pointed out above that one of the first changes, when seed quality deteriorates, is a degradation of the cell membranes. This can occur both during storage and mechanical damage. Such seeds have a low ability to restore cell membranes. Placed in water, when it is absorbed, it begins to release substances from the cytoplasm in the medium. Sugars, amino acids, fatty acids, proteins, as well as potassium, magnesium, sodium, manganese and calcium ions are released from the seeds.

These substances have electrical properties and carry an electrical charge that can be measured conductometrically. However, moisture and seed size affect the properties of the solution. According to Ching and Schoolcraft (1968), an increase in the release of substances during water adsorption depends to a large extent on the degradation of cell membranes and the lack of control or management of the permeability. It is indicated that when such seeds are sown and germinated under field conditions, these secretions can be a favorable condition for the development of microorganisms (Panayotov, 2015; Marco-Fliho, 2015; Panayotov 2016).

Marco-Fliho (2015) examines the historical development of this test. He points out that Fick and Hibbard (1925) were the first who express at the opinion that low germination is related to the release of substances during seed soaking. About thirty years later, Priestley (1958) reported that the electrical conductivity of a water solution in which seeds were soaked was inversely proportional to their germination rate. For the first time, however, the possibility of using this discovery to use as a test of vigor was proposed by Matthews and Bradnock (1967), who developed a methodology and a way to evaluate the emergence of seedlings of pea seeds. This is one of the first tests included in the International Rules for Seed Testing of ISTA for garden pea seeds.

Vegetable seeds are characterized by their small size, they quickly change their humidity and are often subject to longer-term storage. In this sense, the conductivity test is often applied to the seeds of these species. Through the use of different methods, a very close correlation with field germination of carrot and pepper seeds was reported using this test, achieving rapid and real information on seed status (Andrade et al., 1995; Torres, 1996).

However, there are some conflicting opinions regarding the applicability and reliability of the conductivity test. Ratajczak and Duczmai (1991) and Prusinski and Borovska (1993) think that this test has less opportunity to predict seed vigor and has very pronounced differences between cultivars, making it difficult to unify. On the other hand, Dong et al. (1998) observed that in increasing the storage time of onion seeds the vigor decreases as well as the electrical conductivity increase and germination and field emergence decrease, but they also reported a negative correlation between electrical conductivity and emergence.

This indicates that the application of the conductivity test is still debatable. Aladzhadjiyan and Panayotov (1994) conclusions are close to this opinion, that during long-term storage of tomato seeds, germination decreases and electrical resistance decreases, i.e. electrical conductivity increases. These authors also found some controversy in pepper seed storage. In support of the specificity of the data obtained is also the study of Ozoban and Demir (2002) in pepper and watermelon seeds, pointing out that the conductivity test does not always provide consistent results to relate to germination and emergence.

In addition to seed storage, studies have been carried out that reveal that this test is also suitable for assessing the potential of seeds during their development. Panayotov and Aladzhadjiyan (1999) and Aladzhadjiyan and Panayotov (1999) reported that as pepper and pea seed development progressed, there were changes in electrical conductivity that were strongly correlated with their germination. They hypothesize that as the growth, formation and maturation of the seeds progresses, the integrity of the cell membranes improves and thus the release of substances from them decreases, which significantly affects the conductivity. According to the authors, this method is suitable to be applied to establish the stage of seed development.

An electrical conductivity test is defined as belonging to the group of rapid tests and is essentially biochemical. The advantages of this test are also that it is inexpensive, numerically measurable, and often the results are accurate, as well as being easy to implement. However, some disadvantages should be pointed out that it shows data only for the seeds placed for analysis and only by assuming that all seeds in a lot will show this character, but it should not be forgotten that each seed is specific with its own characteristics.

Test of controlled seed deterioration

Such a test is well suited to be applied to vegetable as well as small-seeded species. Black et al. (2008) described its essence, pointing out that it aims to identify differences in vigor in small-seeded crops such as vegetables. Basak et al. (2006) indicated that a number of researchers such as Matthews (1980), Powell and Matthews (1981), Powell and Matthews (1984a) and Powell and Matthews (1984b) applied this test to predict field germination and seed storage of some vegetable crops such as cabbage, onions and carrots.

The presumption is that the seeds of the studied batches are under the same conditions, thus aging and deterioration of their potential can be achieved. It starts with equalizing the humidity of the seeds. The seeds are placed on moist filter paper and the moisture in them is periodically measured using the weight method until the required moisture is reached. To balance and evenly distribute the moisture in the seeds, they are placed in impermeable, hermetically sealed envelopes (sealed) of aluminum foil and kept overnight or, depending on the culture, for a longer period (for example, up to 5 days) at a temperature of 5-10° C. After which, for 24 hours, they are placed in a water bath with a temperature of 45°C. To reduce the temperature immediately after removal, the envelopes are immersed in cold water. The seeds thus deteriorated are tested by the standard germination test.

Basak et al. (2006) recommended the application of the controlled deterioration test for 24 hours at 22% seed moisture as a routine method for predicting germination and storage duration of pepper seeds. The effectiveness of the test is also confirmed by the results of Rodo and Marcos-Filho (2003) for onion seeds brought to a humidity of 24%.

Of interest are the experiments that have been carried out by Eksi and Demir (2011) to reduce the time to conduct this test. When assessing the vigor of onion seeds, they modify the standard controlled deterioration test so that seed moisture, equilibration and aging are completed within one day, and the standard germination test is completed within four days. They found a very strong correlation between the data obtained from the traditional test and the new, modified one.

By its nature, this test approaches the principles of the accelerated aging test. A disadvantage of this test is that it is not fast, it requires a long preliminary preparation of the seeds and then a germination test. In addition, as Matthews (1993) points out, the required seed moisture and aging duration should be established for each crop. In this direction is the opinion of Rodo and Marcos-Filho (2003) that it is a laborious test needing prior standardization of seed moisture

A perspective for scientific researchers working on the controlled deterioration test is to direct their studies to create and modify the test to shorten its duration. Nevertheless, this test has significance and provides high sensitivity for the vigor evaluation of vegetable crop seeds.

Cold test

Among seed scientists, this is one of the oldest tests for assessing seed potential, so it should be given the attention it deserves. With it, the seeds are in a state of stress. The test setup consists of using a strip of soil in filter paper or directly in soil, where for a certain period the seeds are placed at low temperatures. The presence of low temperatures on the one hand and microorganisms on the other create stressful conditions for water absorption. After passing a certain period in which seeds are in an environment of low temperatures, they are placed at optimal temperatures so that they can germinate normally (Panayotov, 2015).

Marcos-Fliho (2015) points out that although this test was mainly created for maize seeds, it is also suitable for vegetable crops such as carrot, eggplant, beans, lettuce, and onion. By using soil, it is possible to evaluate more sensitive seed lots compared to the standard germination test. The stressful conditions to which seeds are subjected are created by the combination of low temperatures, high environmental humidity and pathogens. The stress of suboptimal temperatures is due to fact that, when water is absorbed, the integrity of membrane systems is degraded and substances leak out of cells, including sugars. The restoration of cell membranes under such conditions is quite difficult and this increases the discharges from the seeds.

On the other hand, in the presence of soil, the microorganisms also act as a stress factor. Thus, the rate and percentage of germination and emergence are lower and the vigor of the given lots are evaluated by the surviving seeds in such an environment. According to him (Marcos-Fliho, 2015), research on this test began as early as 1926-27 with the experiments, respectively, by Dickson and Holbert (1926) on infecting seedlings in soil and by Alberts (1927) on the effectiveness of fungicides. In 1940, it was already introduced for corn seed control, but according to the author, as TeKrony (1983) pointed out, it only became popular in Europe in 1976.

Good results, near to germination and emergence under field conditions of onion seeds were reported by Piana et al. (1995), treating the seeds for seven days at a temperature of 10^0C and under room conditions.

Although many laboratories emphasize the usefulness of this test in vegetable crops, there are also known failures. Its negative features are the differences in individual soils, their humidity and pH, and the presence of different pathogens, which is why very often differences in data are obtained and there is a lack of repeatability, and this prevents its standardization. One way to overcome these difficulties is to include perlite or vermiculite in the medium, but using conditions very close to real life.

Tetrazolium (TZ) test

It is one of the most widely used tests that provides substantial opportunities for vigor assessment without the need to wait for the results of a standard germination test. The beginning of this test was set in 1940, by the German Prof. Georg Lakon. To distinguish living from dead seeds he first used selenium salt, but the results were not reliable. Subsequently, he turned to the application of tetrazolium salt and found its higher efficiency in staining the tissues. According to Marcos-Filho (2015), even before that, as Moore (1969) points out, attempts to assess seed viability by applying dyes were conducted in 1922 by Turina in Yugoslavia and in 1925 by Neljubow in Russia.

The test is based on distinguishing between living and dead tissues in the embryo and whole seed based on their relative rates of respiration when hydrated. During respiration, the activity of many enzymes increases, but dehydrogenase enzymes are important here, which are an indicator of the rate of respiration and vitality of the seeds. First, the seeds are placed to swell in a moist medium and all tissues are fully hydrated. Then they are placed in a 1% solution of tetrazole (2,3,5,-triphenyl tetrazolium chloride or bromide) in a thermostat at a temperature of 35^0C for about two hours. For some species, it can be longer. This duration is important, because with a longer stay, the results may not be real, and the vitality may be overestimated. Many species of seeds can absorb the solution when whole. However, there are those in which, for the solution to enter, it is necessary to break their integrity in a mechanical way (Copeland and McDonald, 2001).

For the seeds of some vegetable crops, the method of preparation is well known. Bean seeds are soaked at 20^0C for 15 hours or at 30^0C for three hours, with the help of a needle the coat is peeled, and the exposed seeds are washed with water and soaked in the tetrazolium solution for 16-24 hours. The seeds where the embryo root or cotyledons are well-colored are defined as viable. A similar procedure is recommended for pumpkin seeds. After soaking in water at a temperature of 20^0C for 20 hours, the coat of the swollen seeds is removed with a needle and soaked in the tetrazolium solution. Fully colored seeds or those in which only the tip or at most 1/3 of the length of the radicle is not colored, as well as such seeds that have small uncolored spots on the cotyledons at the opposite end of the radicle or have no uncolored spots near the radicle are considered viable. (Panayotov, 2015).

The mechanism of action is caused by the fact that the colorless tetrazolium solution is absorbed by the seeds, by means of the reduction process it is mixed in the tissues of living cells and takes up the hydrogen that is released by dehydrogenase. As a result of the dehydrogenation of tetrazole in living cells, a stable, insoluble red-colored substance called triphenylformazan or just formazan is formed. In contrast, no such substance is formed in dead tissues. This is how living and dead tissues are distinguished. Above all, depending on the place of coloring (topographical coloring) and the intensity of the red color, vitality is also determined. In this way, experienced analysts by color divide the seeds into categories from strong to weak. It should be noted that in addition to live seeds that are fully colored and dead that are colorless, partially colored seeds are also observed. The location of staining, in the embryo or the endosperm, and not so much the intensity of the color is of greater importance for the viability assessment (Copelan and McDonalds, 2001; Panayotov, 2015). The location, the degree of coloring and the presence of spots in the tissues as indicators of the vital state of the seeds are also highlighted by other authors, and depending on this, some divide the seeds into categories such as vigorous, viable but not vigorous or non-viable (Marcos-Fliho, 2015).

The importance of the location of staining was also emphasized by Sales et al., (2022). Applying the tetrazolium test to kale (*Br. oleraceae,* var. *acephala*) seeds, they mainly observed the staining in the places of the seed that are important for germination. The seeds depending on the place of staining are grouped into viable and non-viable groups and also subdividing them into different categories depending on the place and intensity of staining (Figure 6).

This test is very promising for establishing the level of vigor. Andrade et al. (1995) indicated that it could determine the viability of carrot seeds in 3 hours, while the standard test required about 14 days. In vegetable crops, a greater opportunity for its application is outlined in species from *Cucurbitaceae, Brasiicacea* and *Fabaceae,* i.e. seeds whose coat peels easily.

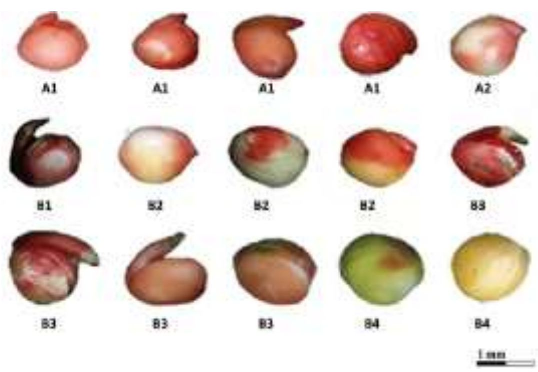


Figure 6. Classes for determination of viability in kale seeds by the tetrazolium test: Category A (viable): A1 – well-developed embryonic structures, intact and with pink staining, and A2 – less than 50% of the embryo unstained, without reaching the embryonic axis, and tissue with normal and firm appearance, Category B (not viable): B1 – embryo with intense red staining; B2 – more than 50% of the cotyledons unstained; B3 – embryo axis region unstained; and B4 – embryo completely unstained (by Sales et al., 2022)

Positive aspects of the Tetrazolium test are that it is fast, belongs to the group of biochemical tests and most importantly provides accurate and precise information about seed vigor and has a strong positive correlation with their real field performance. It is also very suitable for vegetable crop seeds, but further studies are required to establish species-specific protocols.

A disadvantage is the subjective factor, the assessment by the evaluator (analyst) regarding the degree and place of staining. It requires preliminary preparation of the seeds, which can cause difficulties from a technical point of view in the case of small-seeded species. A well-trained staff is also needed. Phytotoxicity can also occur with incorrect application.

Seedling vigor classification test (Test by seedling morphological behaviors)

The morphological characteristics and behaviors of seedlings have long been used to evaluate the physiological potential of seeds. Seeds with higher vigor absorb more fully the reserve nutrients of the seed and redirect them better to the growing parts of the embryo, which has a positive effect on the growth and development of the seedlings. One of the first signs of seed deterioration is the slower development and protrusion of the embryo root. The strength of this deterioration is directly proportional to the duration of the sprouting phases, especially the second phase, the so-called “lag-phase”, i.e. depending on the level of deterioration of the seeds, the time required for metabolic recovery of the damage on the seed will also depend before it continues to germinate. The speed of germination and uniformity of seedling development are also important in this regard (Marcos-Fliho, 2015).

To determine to what extent the individual parameters and properties of seedlings are suitable for assessing vigor, several studies were conducted, including the determination of germination, germination rate, emergence, development and formation of dry weight of seedlings, embryo root development, and others. Special attention is paid to the rate of germination, as it is a much more sensitive indicator than the percentage of germination and therefore more suitable for vigor determining.

Actually, this test expands the standard germination test by additionally allowing seedlings to be classified as strong and weak, but also as abnormally developed. In this direction, Wellington (1970) also made an extremely large contribution, studied and established deviations from the normal development of seedlings of many crops, and his catalog is widely used by researchers in the field of seed science.

In conducted the experiments in the Department of Horticulture of the Agricultural University-Plovdiv, Bulgaria, experiments on the storage of pepper seeds for ten years, Panayotov (2010a) found that the duration of storage has a strong influence on the development of seedlings. Until the 4th year, the deviations are relatively small, after which they begin to increase significantly to reach 90% and above 90% in the tenth year (Table 2). In other studies, with storage of cape gooseberry seeds (Table 3), Panayotov (2023) reports that, despite reported high germination, seedlings with deviations from normal development are observed from the second year, and with loss of germination, their percentage reaches approximately 40%. More common abnormalities are unopened cotyledons, lack of hairs on the radicle, underdeveloped or short radicles, and lack of lateral branches. Correlations with results of another vigor test are strong and positive, indicating that seedling development and its changes are a suitable way to assess vigor. Such an opinion about the importance of the development of seeding and its relationship with vigor is also expressed by Bewley et al. (2013).

The positive side of this type of test is that they do not require specific and additional equipment, the same approaches and terms are used as for the standard germination test. Disadvantages and difficulties originate from the circumstance, that it is not a quick test. To be able to analyze the seedlings, to categorize them into strong and weak, very good knowledge is required. Here, too, there is a subjective factor of the analyst's views and capabilities.

Close to this test is the *Seedling growth rate test (Test of dry bio-matter).* It is based on the idea that new biomass is synthesized when the seeds germinate. These substances are directed for the active growth of seedlings and dry weight is formed. The dry weight of normally formed seedlings is determined by the standard method in an oven at a temperature of 80^0C for 24 hours. The results of these tests are considered to correlate well with vegetative development under field conditions. A disadvantage is that equipment is required and above all those minor differences in humidity and light affect seedling growth. Further standardization is also required for individual species, as seedling growth is genetically controlled.

Test of "Initial vegetative productivity of the vegetable seeds"

This is a new method developed by N. Panayotov in the Department of Horticulture, at the Agricultural University-Plovdiv, Bulgaria (Panayotov, 2013; Panayotov, 2016). The concept of "Initial vegetative productivity" means the development of the seedling fresh weight from the seed, based only on the reserve substances contained in the seed itself, thus eliminating the use of nutrients from the germination medium and so the development of seedlings is only based of the capabilities of the seed, i.e. of the nutritional ingredients available in it. This is also the main hypothesis on which the test was developed. Apart from the fact that the development of seedlings is only based on the reserve substances in the seed, as an additional factor and somewhat stressful conditions, the possibility of seeds from batches with better vigor to overcome the resistance of the sand cover more easily and quickly is also included, to appear on the surface and as a result of most nutrients to form a larger vegetative weight.

Table 2. Pepper seedlings deviation after storage (%) (Panayotov, 2010a)

Period of storage (years)	Kurtovska kapia 1619		Bulgarski rotund	
	(%)	Type of deviation and percentage to total number of deviations	(%)	Type of deviation and percentage to total number of deviations
1	10.0	**K**-40.0; **E**-60.0	0.0	
2	13.33	**E** – 71.42; **K**-28.56	3.33	**H**-40.0; **K**-60.0
3	16.67	**H**- 71.4; **E**- 28.6	10.0	**K**-33.33; **H**-66.66
4	13.33	**E**– 66.1; **H**– 22.1; **K**– 17.8	13.33	**E**-100.0
5	30.0	**K**-42.85; **C**- 57.15	16.67	**R**-91.0; **K**-9.0
6	23.33	**R**-66.6; **H**-33.33%	33.33	**E**-100.0
7	33.33	**K**-25.0; **H**-75.0	30.0	**H**-50.0; **K**-50.0
8	76.67	**E**– 52.3; **H**–37.3; **K**–10.4	70.0	**H**-100.0
9	70.0	**E** – 48.3; **H**– 32.3; **K**–19.4	70.0	**K**-50.0; **H**-50.0
10	90.0	**E**- 68.3;**H**–12.3; **K**– 19.4	96.67	**H**-88.12; **E**- 11.88

E-underdeveloped embryo root; **H**-without hypocotyls; **K**-without hairs ;
R- lack of root branches; C-unopened cotyledons

Table 3. Deviation of cape gooseberry seedlings after storage (%) (by Panayotov, 2023)

Year	Plovdiv		Obrazec 1	
	%	Type and % to the total number of deviation	%	Type and % to the total number of deviation
1	9.5	HR-52.2; H-47.84	10.8	BR-38.8; H-51.2
2	16.6	SR-28.8; BR-35.2; C-36.0	15.7	H-55.3; C-44.7
3	19.5	BR-48.8; C-41.2	20.9	SR-42.8; BR-27.2; C-30.0
4	22.4	HR-45.5; BR-30.0; C-24.5	24.6	SR-41.5; H-26.5; C-32.0
5	28.3	H-42.3; C-57.7	31.3	HR-48.8; C-41.2
6	36.8	SR-35.5; H-36.6; C-27.9	38.6	SR-25.0;BR-15.5;H-20.5;C-39.0

SR-Short embryo root, BR- Lack of branches in embryo root, HR-Lack of hairs on the embryo root, H-Lack of hairs of hypocotyls, C-Unopened cotyledons

The test is based on sowing the seeds in physiologically clean river sand, with a particle size of 3 mm, sterilized for 4 hours at 105^0C, placed in disinfected containers, in a layer of at least 10-15 cm, levelled and compacted. The seeds are sown at the same depth, depending on their size and species, density depends on the crops, but usually with a distance between the seeds three times their largest size, which is its length. The covering layer is of the same sand of equal thickness, levelled. Sowing 100 seeds in 4 replicates is recommended. It is watered with distilled water with a pH of 7-7.5, in the same quantities for all replicates. The containers are placed under conditions of temperature and humidity, depending on the prescriptions for the respective species for seed germination and growth, with an assured light regime day/night, according to the real conditions, in the perception of the moment of sowing the seeds of the field in a given region. All the time, the humidity required for the species is maintained (Figure 7).

Observations and reports are carried out when there is a visible stoppage of development and lack of growth of seedlings, growth retardation. This usually occurs at the stage of fully formed cotyledons or a well-formed first pair of true leaves and the beginning of a third. The number of seedlings is counted in two groups - with only cotyledon leaves developed and the second group - with at least the first pair of true leaves developed, and their percentage is determined depending on the total number of sown seeds. On 15 plants of each replicate, carefully removed with a spatula to the depth of the base of the container, the adhered sand was washed, and absorbed with filter paper and the weight of seedlings was measured separately for each of the two groups on a scale accurate to the second mark. The data are averaged for each replicate and then averaged for each of 4 replicates and the biomass developed from one seed is determined.

The vigor index (Y) in percentage is determined by the equation:

$$Y = \frac{(m_1 p_1) + (m_2 p_2)}{100}$$

m_1 - weight of the plant in the cotyledon phase (mg)
p_1 – percentage of plants in cotyledon phase
m_2 - weight of the plant in the first pear og true leaves phase (mg)
p_2 - percentage of plants in phase one true leaf

Additionally, in the final assessment of vigor for individual batches, the time in days that was required for seedling development is indicated. Thus, vigor is determined as a result of morphometric measurements and phenological observations.

Figure 7. Experimental setting for determination of vigor of pepper seeds trought the Test of Initial Vegetative Productivity (Panayotov, original)

This methodology belongs to the group of direct, morphological tests, through its application a better comparative assessment is achieved between individual batches. The advantages are that it is cheap, as it does not include special and expensive equipment, it is accessible - no additional training of personnel is needed, and the results are real and accurate, and only by one experiment are observed three main factors - resistance of the sand, seedling development based only on the nutritional components in the seed and the time required for seedling development. Also, the results are presented numerically. As a disadvantages, this test is not fast and requires an area for placing the containers, as well as some influence of the subjective factor of the analyst to evaluate the end of seedling growth.

The reliability of this test is confirmed by the experiments carried out with naturally aging pepper seeds aged 1-10 years, as well as with three batches of seeds of the same crop with different origins of seed production (Panayotov, 2013). The aging of seeds causes a decrease in the biomass formed (Table 4). The sensitivity of the test is also evident at the stage of leaf formation in the tested variants. Seedlings, with well-developed true leaves, respectively, and cotyledons are counted until the third year, and their percentage compared to one-year seeds is lower at 20-30%. Formation of seedlings with only cotyledons is reported up to 7-8 years, and the reduction of their percentage compared to those from seeds in the year of production is between 50-60% depending on the variety. After this period no seedlings develop at all.

For one-year seeds, the vigor index for initial vegetative productivity is around 60-65, and after 7-8 years of storage, it drops to 3. The presence of these large differences indicates that this test has very good sensitivity and is suitable for vigor assessment. Moreover, when compared to other tests, a similarity in trends is found (Figure 8). The obtained curves between this test and for the other two tested (according to Elliot, 2001) and for one seed (Panayotov, 2010b) are similar. At the same time, a decrease in the test data for initial vegetative productivity occurs significantly earlier than those for germination and for the other studied tests, and it once again confirms its higher sensitivity. With a decrease in germination from the initial values by 20-30%, the vigor decreases by approximately 70%, and in the other two tests by 30 - 50%. All this shows that the decrease in vigor according to this test occurs earlier and is an excellent prerequisite for predicting seed deterioration.

Table 4. Vigor by Initial vegetative productivity of storage pepeer seeds (Panayotov,2013)

Years	Kurtovska kaipa 1619					Bulgarski rotund				
	Seedlings with cotyledons		Seedlings with first leaf		Vig-or index	Seedlings with cotyledons		Seedlings with first leaf		Vig-or index
	Weight (mg)	%	Weight (mg)			Weight (mg)		Weight (mg)	%	
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0	0.0	29.4	11.2	0.0	0.0	3.29
7	32.3	10.3	0.0	0.0	3.32	32.2	20.4	0.0	0.0	6.56
6	38.2	40.3	0.0	0.0	15.39	36.5	41.2	0.0	0.0	15.03
5	42.5	42.8	0.0	0.0	18.19	42.0	45.6	0.0	0.0	19.15
4	46.7	48.2	0.0	0.0	22.50	52.6	48.9	0.0	0.0	25.72
3	51.0	51.2	110.8	6.3	33.09	60.6	55.9	137.2	7.0	43.47
2	57.5	59.8	132.2	10.8	48.66	67.1	60.0	156.3	10.0	55.89
1	62.8	63.8	153.5	13.5	60.78	74.5	60.0	170.5	12.3	65.67
LSDp= 0.05	3.53	8.47	13.54	2.41		3.53	6.99	2.46	2.24	

Vigor data for seeds from batches of different origins showed considerable variation between cultivars tested, with vigor index values ranging from 48.51 to 70.19 depending on cultivar, seed origin and seedling development (Table 5). This again shows the higher sensitivity of this test and obtaining real results. Also, from Figure 9 observed a trend match with other tests, as well as a preceding decrease in vigor versus germination and the results of other tests, proving the advantages of the Initial vegetative productivity of the vegetable seeds test and its sensitivity.

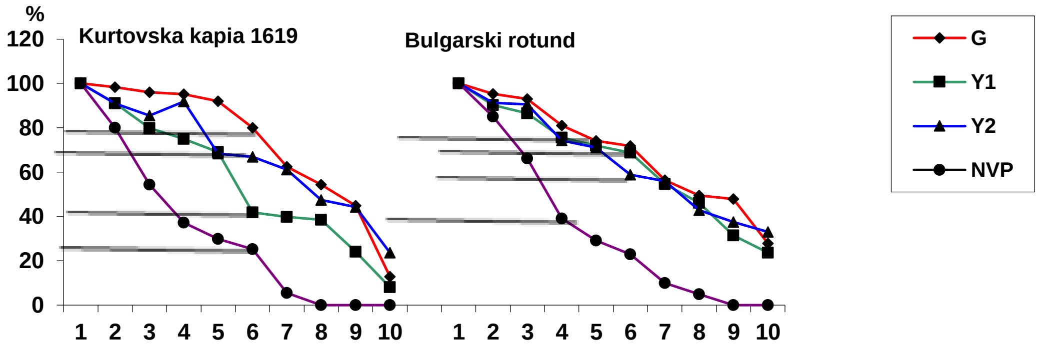


Figure 8. Changes of germination (G), vigour index of samples (У1), vigour index of one seeds (У2) and vigour index by initial vegetative productivity (IVP), (1-10 age of pepper seeds as Table 4) (Panayotov, 2013)

Table 5. Changes of the vigour of pepper seeds with different origin, by initial vegetative productivity of seeds (Panayotov, 2013)

№	Cultivars, Origin	Seedlings with cotyledons		Seedlings with first leaf		Vigor index
		weight (mg)	%	weight (mg)	%	
I	Kurtovska kaipa 1619, Vardim	78.4	65.1	134.2	5.9	59.03
II	Kurtovska kapia16191, Striama	74.3	65.3	0.0	0.0	48.51
III	Kurtovksa kapia 1619, Plovdiv	86.6	55.6	158.4	5.6	57.02
IV	Bulgarski rotund, Vardim	88.3	68.2	166.2	6.0	70.19
V	Bulagarski rotund, Striama	76.8	56.8	142.3	10.6	58.70
VI	Bulgarski rotund, Plovdiv	81.2	60.4	156.2	8.8	62.79
VII	Delicates, Vardim	74.4	65.1	150.2	6.8	58.64
VIII	Delicates, Striama	69.6	57.7	128.6	10.0	53.01
IX	Delicates, Plovdiv	77.7	64.1	0.0	0.0	49.80
	LSD p=0.05	3.1	2.0	5.75	2.37	

Non-invasive tests.

Evaluation of vigor without destruction of the seeds, i.e., so-called non-invasive tests, finds more and more application in increasing research in this area. Their importance increases because they are usually fast, very often automated, and mainly involve physical analyses of individual seed properties that are strongly correlated with vitality and vigor. In most cases, these are rapid tests and information on vigor is obtained at the time of examination. However, the use of these tests is still weak, insufficient and limited.

The application of UV light reveals the possibility of a non-destructive way of determining vigor based on the fluorescence. Placed under such light, the seeds react differently depending on their degree of vitality. Seeds that are vitality and have high vigor do not fluoresce. Evidence for the appropriateness of this test and the accuracy of the results Lee et al. (1997) found in their experiments with Chinese cabbage and radishes. Non-fluorescent i.e. live seeds of these two crops, in the investigation of this test, examined by the standard germination test showed germination rates of 97-98% and 84-87%, respectively. Radish seeds that had fluoresced, i.e. those that could be defined as dead, had a germination rate of only 4-7%.

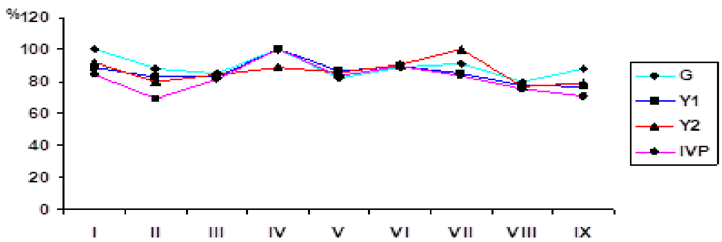


Figure 9. Trend of germinability (K), vigour index of whole sample (У1), vigour index of one seed (У2), initial vegetative productivity (IVP) of pepper seeds with different origing. (I -IX as in Table 5) (Panayotov, 2013)

The fluorescence has also been used by other researchers to assess vigor and seed sorting (Cicero et al, 2009). Jalink et al. (1998) reported a rapid and relatively accurate method for determining maturity, vigor and viability of whole seeds based on chlorophyll fluorescence data. The correlation between reducing the green color of the seeds and increasing their vitality is very strong. Vegetable crop seeds that showed a weak chlorophyll fluorescence signal had higher vigor and developed normal seedlings. The opposite is true for seeds with low germination, where the fluorescence signal is much stronger.

Dead seeds were found to release significant amounts of sinapin, amino acids, and proteins, while healthy and vigorous seeds with high vigor released minimal amounts of these constituents. On this basis, Lee et al. (1995) developed a new non-destructive test for vigor assessment.

Another guideline for non-destructive testing is the use of Near-infrared spectroscopy (NIR) combined with multivariate seed calibration. In advance, the seeds from the individual lots are separated and sorted into two groups: viable and non-viable using X-ray sorting. The NIR reflectance spectra are recorded using a fiber optic probe, and through various corrections (multiplicative and orthogonal) the systematic noises in the spectra are removed. The accuracy of seed separation in both groups using these models was almost 100%. The main source of spectral variation is seed moisture, but lipids and proteins also give their reflection to accurate separation. Predicting the physiological status of seeds by non-destructive NIR spectroscopy is rapid and has a high potential to eliminate non-viable seeds (Soltani et al., 2003).

Image diagnostics of seeds

X-ray vigor test.

The possibilities offered by radiographic studies to assess the potential and vigor of seeds are promising. Vigor tests developed on this basis are completely non-destructive and, most importantly, they are fast. The studies in this scope are new, they are implemented through the inclusion of smart technologies and are very promising due to the accuracy of the results and obtaining the information in a relatively short time. They mainly focused on the anatomical status of the seeds and above all the tissues of the embryo and endosperm and at the same time also showed deficiencies in the structures of the seeds, related to their vitality.

The possibilities of X-ray studies also have been evaluated by ISTA (2019), as in its rules it is emphasized that this method distinguishes full, empty, insect-attacked or physically damaged seeds by anatomy-morphological characteristics, as well as the ratio between them, visible on the radiographs. Suitable for imaging of small objects, such as the seeds of vegetable crops, are low energy (shorter wavelength) X-rays, low voltage mode (low kilovolt apparatus) which improves resolution and keep in mind that high mAs overexpose (darkenc) the image or these are the so-called "soft" X-rays apparatus. Exposure time is also important. The basis of this method is that different seed tissues, depending on their thickness and density, absorb X-rays to a different degree. The evaluation of on the X-ray picture is based on the internal anatomy of the seeds, dividing them into the groups indicated above.

The disadvantages of these tests are that they are expensive, require additional specialized equipment, and require prior training, both for preparing the X-ray and evaluating the results.

Chavagnat and Bastien (1991) emphasized the positive aspects of X-ray analyses that there was a very good correlation with high significance between the seed germination test and radiography. In this technique according to Burg et al. (1994), with the help of X-rays in tomato seeds, the anatomical and morphological changes in the seed sprouts, as well as the reasons leading to a decrease in vitality, primarily related to the status of the embryo and endosperm have been revealed.

According to Gomes-Junior et al. (2012) X-ray densitometric tests of seed status are non-destructive and should find wider application, considering, that assessment of seed anatomy is accurate and its relationship to germination and vigor is significant. An X-ray study of the internal anatomy of pumpkin, cantaloupe, and watermelon seeds a typical density profile of specific areas within the seed that could identify the causes of poor germination and low vigor of each seed has been registered.

To obtain a precise image Dos Santos et al. (2009) recommended a radiation exposure regime of 20 Kv for 1.5 min which revealed very well the anatomical profiles of tissues in the embryo and endosperm. According to the radiographic data, the seeds can be classified into the following groups by signs related to germination: full seeds, empty seeds, those with disturbed embryo and embryo cavities, and seeds with endosperm and inconspicuous embryos. The potential capabilities and reliability of the information from this test are demonstrated by the high correlation found with the data from the standard test.

Downie et al. (1999) established by X-ray analysis, the anatomical condition during individual stages of tomato seed development using a similar radiation regime of 15 kV and 2.5 mA for 1.5–3.0 min. X-rays are examined under a dissecting microscope, thanks to which this analysis makes it possible to establish differences in the structure and anatomy of the seeds associated with manifestations of vigor. As a result, three classes of seeds are formed: with an indistinguishable space between the embryo and the endosperm; with limited but noticeable space between the embryo radicle and the micropylar tip of the endosperm and/or cotyledons and endosperm; with a large space between the tip of the embryo root and the micropylar endosperm, and between the endosperm and the cotyledons.

The results of the X-ray analysis were also confirmed and proved on scanning electron micrographs of the same seeds (Figure 10). The seeds of the third group were found to occur more in mature fruits and show a distinct point at the most distal end of the root tip, but it is important that with them the required puncture force is smaller. Although the established availability of free spaces can serve as a diagnostic of seed development, the authors point out that further research is needed to create a predictive model.

Digital imagine analysis

The state-of-the-art techniques for determining seed potential and vigor are various computer seed imaging approaches. In this field, extremely active work has been done in the last two decades. Through them, much more extensive and accurate information about the morphology and biology of the seeds is obtained. This allows the improvement of the sorting and qualification processes of the individual lots. The automatically recorded data on some basic morphological characteristics of the seeds, such as size, shape, and color, related to germination evaluation, successfully complement those of the standard germination test and also allow the identification of cultivars and weed seeds.

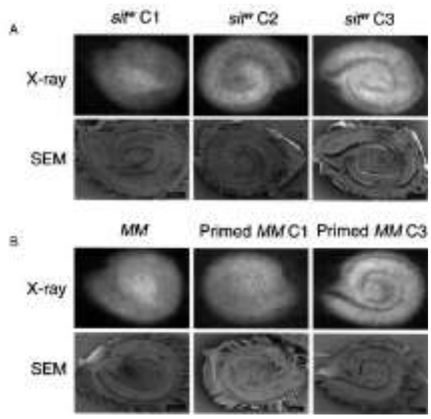


Figure 10. X-radiographs (upper panels) and scanning electron micrographs (lower panels) of tomato seeds (by Downie et al., 1999)

These tests are also associated with the application of computed tomography to study the internal morphology and function of the seeds. Developments are growing to integrate data from human assessment of seed status and the creation of computer vision in laboratories. Recently, a great deal of attention has been paid to interdisciplinarity in research, by combining information from digital images with that obtained from genomic and proteomic analyses, contributing to more accurate characterization of seeds using non-invasive methods and obtaining a more comprehensive view of seed research (Dell'Aquila, 2007).

Constantly looking for new opportunities to standardize tests, increase their accuracy, shorten the time for conducting them and improving the repeatability of information. For all this, the introduction of computer technology for establishing the status of the seeds provides very good opportunities. Research and implementation of these methods will increase. The results obtained so far on the images of seeds and seedlings are extremely significant and interesting, computerizing their morphological features and providing a direct, computerized assessment of vigor (Marcos-Fliho, 2015).

Among the first, according to Marcos-Fliho (2015), to introduce computer-based image analyses of seeds and seedlings based on embryo root length in carrot and lettuce was McCormac and colleagues (McCormac et al, 1990), as well as Howarth and Stanwood (1993) also with lettuce. Indisputably the greatest advance came when developed by Sako et al. (2001) automated the Seed Vigor Imaging System (SVIS®) with which they assessed lettuce seed vigor. In the images of seedlings, with the help of special software, the individual parts are identified and marked, and after computer processing, the length of the embryo root, the hypocotyl, the ratio between them and the entire seedling, as well as the vigor and uniformity of the seedlings are determined. Subsequently, this system was also applied to several vegetable crops, such as cucumbers and eggplants. Computerized image analysis systems are becoming increasingly sophisticated and based on SVIS®, the Seed Vigor Automated Analysis System (Vigor-S) was created, improving the efficiency and objectivity of the obtained vigor information. (Marcos-Fliho, 2015). Marcos-Fliho (2006) scanned 3- and 4-day-old melon seedlings and, based on the computer images, established vigor, such as the obtained results for vigor index, uniformity, and seedling length are comparable to data from tests already adopted.

Applying a number of tests, including the Seed Vigor Imaging System (SVIS), Peñaloza et al. (2005) points out that the SVIS data are objective and well distinguish the strong from the weak lots, and at the same time are in good correlation with those obtained from the salt saturate accelerate aging test and this gives them reason to recommend SVIS for a vigor assessment.

In addition, Zhang et al. (2022) also included multispectral analysis for vigor estimation as a new rapid and non-invasive method with high sensitivity and accuracy. 19 different wavelengths are used, and the information from morphological studies and that from multispectral for seeds of different maturity and age are analyzed and it is concluded that through multispectral imaging and multivariate analysis, vigor, viability, germination percentage can be accurately predicted of the seeds. To obtain the images, they use the multispectral imaging instrument VideometerLab4 (Videometer A/S, Herlev, Denmark).

The positive sides of this type of tests are that they are non-invasive, fast and with good accuracy, but the disadvantages are that they are expensive, involve specialized equipment, usually require the presence of large databases and require very good preliminary training of personnel.

RECOMMENDATIONS AND TASKS FOR FUTURE RESEARCH

Determination of seed vigor of vegetable crops is taking on a wider scope and it is gaining more and more importance, both in scientific research and in seed production, qualification, marketing and use of sowing material. Despite the great activity carried out up to this point, there are important questions that have not yet been fully resolved, and this necessitates the strengthening of scientific research first and, subsequently, for their implementation on a larger scale in practice. Vigor tests should become a routine and well-standardized method for seed quality in vegetable crops where seed production is much more specific, many varieties are heterozygous and more expensive. At the present stage, and in general, the accuracy of vigor tests depends a lot on the species specifics of the seeds. Similar are the guidelines outlined by McDonald (1993), Marcos-Fliho (2015) and Panayotov (2016).

The main tasks and recommendations in front of the the vigor test system of vegetable seeds are:

- Standardization of the existing and widely applied tests, with good repeatability, to eliminate some debatable and contradictory opinions about the meaning of germination, which is an indisputable basis for reflecting the vital potential of seeds and the role of vigor;
- Specifying the procedures, parameters and regimes of application of many of the tests, according to the species specificity of the vegetable seeds. To conduct scientific activities to avoid the indicated disadvanatges in the discussed vigor tests;
- Creation of internationally recognized protocols for the application of vigor tests, since some of them, regardless of their importance, lack them;
- Development of new, modern and perfect tests based on smart and information technologies, expansion of computerization of the process for assessment of vigor. They must meet the accepted requirements, especially in terms of accuracy, their relationship with germination and performance under field conditions, as well as provide the information in a short time (rapid tests) and be associated with minimal financial investments;
- Expanding the training of specialists and analysts, starting from the university education system, as well as through additional courses;
- Deepening interdisciplinary research, including more and more fields of science to create vigor methods and to evaluate the overall potential of seeds;
- Upgrading the understandings and perceptions of the philosophy, essence, biology and meaning of seeds based on new discoveries and information from modern branches of science, such as digital and imaging methods for diagnostics, biophysics, biotechnology and molecular biology in general;
- Inclusion of more broadly of molecular and cellular markers. By in-depth insight into the physiological and biochemical processes of the seeds and above all the protective mechanisms of the seeds related to the change in the activity of some enzymes leading to the deterioration of the seeds. The deterioration of the seeds, as stated above, is associated with changes at the cellular level, of DNA and impaired cell replication, and this is a prerequisite for deepening the molecular analyses;
- Revealing the relationship with changes in protein profiles and especially the possibilities of removing and suppressing free radicals, largely responsible for seed deterioration;
- Applying chlorophyll fluorescence increasingly to identify the status of seeds and especially their maturity, since the presence of chlorophyll in mature seeds limits the manifestations of vigor;
- To look for methods and means for numerical and quantitative expression of the vigor;
- A very important task is the development of predictive models for the changes occurring in the seeds and increasing the predictability of the various processes related to decreasing the seed's quality and opportunities to take proper preventive measures to avoid them;

- Establish regional and international scientific groups to conduct periodic forums for cooperation, create programs, refine the research and improve technical support.

CONCLUSIONS

Undoubtedly, the evaluation of the vigor of the seeds of vegetable crops is of great importance and complements very seriously the understanding of the overall biology and the essence of the seed its upward historical development also speaks for this.

Research on seed vigor is being deepened by incorporating different and modern branches of science to create more accurate and widely applicable tests in many species of seeds.

The understanding of the essence and concept of vigor is improving, both in scientific circles and among growers and traders, who are showing much greater interest and attention to this very important characteristic of the seeds. However, its application at the current stage is not the required level, and the biggest challenge in front of the vigor is the standardization of the tests and they to be applicable in many crops.

Vigor is designed to compare the quality of individual batches of seeds. Starting from the definition of vigor, its assessment should be based on data from research in different scops, Thus, in a much more comprehensive way, the processes taking place in the seed will be understood in depth, and its manifestations in field conditions will be revealed more fully.

For the seeds of vegetable crops, at the current stage the following tests are more suitable and more widely applied: Accelerate aging, Electrical conductivity, Tetrazolium and Initial vegetative productivity of the vegetable seeds, but with great prospects are also X-ray vigor test and Digital image analysis

Deepening the studies on the vigor of vegetable seeds, revealing more and more of its features, and improving the tests and their wider introduction into practice are a good prerequisite for the near future for vigor to become, along with the currently existing criteria, as a mandatory element in quality and standardization of vegetable seeds and to determine the minimum requirements that individual lots must meet worldwide.

REFERENCES

Aladzhadjiyan, A., N. Panayotov. 1994. Physical properties of the seeds of different ages of pepper and tomato. Scientific papers of the Fifth National Symposium with international participation "Physics - Agriculture". Sofia, November 23 to 24. 125-129. (Bg)

Aladzhadjiyan, A., N. Panayotov. 1999. Study ow some physical properties of pea seeds through separate periods of their grow. Crop science. 36 (9): 490 – 494. (Bg)

Alberts, H.W. 1927. Effects of pericarp injury on moisture absorption, fungus attack, and viability of corn. Journal of the American Society of Agronomy. 19: 1021-1030.

Andrade, R. N. B., D. S. B. Santos, V. D. C. Mello, B. G. Santos- Filho. 1995. Germination and tetrazolium tests on carrot seeds stored for different periods. Revista Brasileira de Sementes. 1:108-116.

AOSA, 1991. Association of Official Seed Analyst. Rules for testing seeds. Journal of Seed Technology, 12(3):1-109.

Baalbaki, R., S. Elias, J. Marcos-Filho, M. B. McDonald. 2009. Seed vigor testing handbook, AOSA, Ithaca, NY, USA. 235 pages.

Basak O., I. Demir, K. Mavi, S. Matthews. 2006. Controlled deterioration test for predicting seedling emergence and longevity of pepper (*Capsicum annuum* L.) seed lots. Seed Science and Technology. 34: 723-734.

Bennett, M. 2002. Saturated salt accelerated aging (SSAA) and other vigor tests for vegetable seeds.. In: M. McDonald and S. (Edts.) Proceedings of International Seed Seminar: Trade, Production and Technology. 188-193.

Bewley J. D., K. J. Bradford, H. Hilhorst, H. Nonogaki. 2013. Seeds: Physiology of Development, Germination and Dormancy, 3rd edition. Springer, New York, 358 pages

Black, M., J. D. Bewely, P. Halmer. 2008. The Encyclopedia of Seeds Science, Technology and Uses. CAB International, Oxfordshire, UK. 828 pages.

Broniewski, St., K. Duczmal, J. Korohoda, St. Kowalski, M. Lityniski, K. Strutynska, A. Wilkojc, 1976. Biologia nason i nasiennictwo. Litynski, M. (ed.), Panstwowe Wydawnictwo Naukowe, 1976. In translation: Miroshnichenko, G. N.. Biololgia seman I semenovodstvo. Nikitenko G.F. (ed.) Kolos, Moscow. 463 pages. (Ru)

Brug, W.J., H. Jalnik, R. A. van Zwol, J. W. Aartseand, R. J. Bino. 1994. Non-destructive seed evaluation with impact measurements and X-ray analysis. Acta Horticulture. 362:149-157.

Chavagnat, A., J. C. Bastien. 1991. Determination of seed quality of cupressus-sempervirens l and cupressus-arizonica e greene using roentgenography. Seed science and technology. 19 (1): 139-146.

Ching, T. M., I.Schoolcraft. 1968. Physiological and chemical differences in aged seeds. Crop Science. 8: 407-409.

Cicero, S. M., R.van der Schoor, H. Halink. 2009. Use of chlorophyll fluorescence sorting to improve soybean seed quality. Revista Brasileira de Sementes. 31: 145-151.

Contreras, S., M. Barros. 2005. Vigor Tests on Lettuce Seeds and Their Correlation With Emergence. Cienta Investigation Agriculture. 32 (1): 3-10.

Copeland, L., M. Mc Donald. 2001. Principles of Seed Science and Technology. Chapman & Hall New York. 325 pages.

Crocker, W., J. F. Groves. 1915. A method for prophesying the life duration of seeds. Proceedings of the National Academic Sciences USA. 1: 152-155.

Daniels, S., P. Hysloop. 2003. Almanac of world history. Edition Natioanl Geographic Society. National Geographic. 1: 120.

Dell'Aquila, A. 2007. Towards new computer imaging techniques applied to seed quality testing and sorting. Seed Science and Technology. 35 (3): 519-538.

Delouche J. C., C. C. Baskin. 1973. Accelerate aging techniques for predicting the relative storability of seed lots. Seed Science and Technology. 1: 427-452.

Delouche, J. C., W. P. Caldwell. 1960. Seed vigor and vigor tests. Proceedings of the Association of Official Seed Analysts. 50: 124-129.

Dentcheva, A., D. Klisurska, B. Stefanov, K. Gajdardjiev, A. Nikolova. 1985. Current situation of the question of physiological-biochemical bases of viability and vigour of the seeds. Fiziologija na rastenijata, 7: 100-109. (Bg)

Dickson, J. G., J. R. Holbert. 1926. Influence of the temperature upon the metabolism and expression of disease resistance in selfed lines of corn. Journal of the American Society of Agronomy. 18: 314-322.

Dong, H. Z., R. Q. Gao, Y. P. Yin, S. F. Li. 1998. Study on the physiological-chemical characteristics of Welsh onion (*Allium fistulosum* L.) seed under different storage and package conditions. Scientia Agricultura Sinica. 4: 59-64.

Dos Santos, S.A., R. F. da Silva, M. G. Pereira, J. C. Machado, C. F. Machado, F. M. Borém, V. M. Gomes, O. A. O. Tonetti. 2009. X-ray technique application in evaluating the quality of papaya seeds. **Seed Science and Technology.** 37 (3): 776-780.

Downie, B., S. Gurusinghe ,K. Bradford. 1999. Internal anatomy of individual tomato seeds: relationship to abscisic acid and germination physiology. Seed Science Research. 9: 117–128.

Eksi, C., I. Demir, 2011. The use of a shortened controlled deterioration vigour test in predicting field emergence and longevity of onion seed lots. **Seed Science and Technology.** 39 (1): 190-198.

Elliott, B., 2001. Effect of seed quality on the performance of Argentine varieties in the 2001 regional test. Scientific Report of Part 4 of CARP Project # 2003-02-01-19 of Saskatoon Research Centre, Agriculture and Agri-Food Canada, Conducted at AAFC Saskatoon, Canada.

Evenari, **M.** 1980. The history of germination research and the lesson it contains for today. Israel Journal of Botany (**Israel Journal of Plant Sciences**), vol. 29, issue 1-4: 4-21.

Fick, G. L., R. P. Hibbard. 1925. A method for determining seed viability by electrical conductivity measurements. Michigan Academy of Sciences, Arts and Letters. 5: 95-103.

França-Neto, J.B.; Krzyzanowski, F.C.; Costa, N.P. 1998. The tetrazolium test for soybean seeds. Embrapa Soja, Londrina, PR, Brazil.

Franck, W.J. 1950. Introductory remarks concerning a modified working of the international rules for testing seeds on the basis of experience gained after the world war. Proceedings of the International Seed Testing Association. 16: 405-430.

Gomes-Junior, F. G., J. T. Yagushi, U. L. Belini, S. M. Cicero, M. Tomazello-Filho. 2012. X-ray densitometry to assess internal seed morphology and quality. **Seed Science and Technology.** 40 (1): 102-107.

Heaton, J. 2015. A Brief History of the California Seed Law and CDFA Seed Services., Seed Advisory Board Meeting of CDFA Seed Services, 1-6.

Helmer, J. D, J. C. Delouche, M. Lienhard.1962. Some indices of vigor and deterioration in seed of crimson clover. Proceedings of the Association of Official Seed Analysts. 52: 154-161.

Howarth, M. S., P. C. Stanwood. 1993. Measurement of seedling growth rate by machine vision. Transactions of American Society of Agricultural Engineering 36: 959-963.

ISTA, 2019. International Seed Testing Association. *International Rules for Seed Testing*, Bessersdorf, CH – Switzerland.

Isbouts, J. P. 2009. The Biblical world. Edition Natioanl Geographic Society, part I: 1-23.

Isely, D. 1957. Vigor tests. Proc. Assoc. Off. Seed Anal. 47:176 -182.

Jalink, H., R. Schoor, A. Frandas, J. G. Pijlen, R. J. Bino. 1998. Chlorophyll fluorescence of *Brassica oleraceaL.* seeds as a non- destructive marker for seed maturity and seed performance. Seed Science Research. 4: 437-443.

Jianhua, Z., M. B. McDonald. 1996. The saturated salt accelerated aging test for small seeded crops. Seed Science and Technology. 25: 123-131.

Justice, O. L., L. N. Bass. 1978. Principle and practice of seed storage. Agriculture Handbook number 506. Uniate State Department of Agriculture, Superintendent of Documents, U.S. Government Printing Office Washington, D.C. 289 pages.

Kastler, G. 2005. Seed laws in Europe: locking farmers out. Seedling. Grain 22 Jul 2005 (**https://grain.org/en/article/541-seed-laws-in-europe-locking-farmers-out**)

Lee, S.S., A. G. Taylor, M. M. Beresniewicz, D. H. Paine. 1995. S*ugar leakage from aged leek, onion, and cabbage seeds.* Plant Varieties and Seeds. 2: 81-86.

Lee, S.S., S. B. Hong, M. K. Kim. 1997. Nondestructive seed viability test of Chinese cabbage and radish varieties by sinapine leakage. Journal of the Korean Society for Horticultural Sc. 5: 498-501.

Marcos-Filho, J. 2015. Seed vigor testing: an overview of the past, present and future perspective. Scienta Agricola, 72(4): 363-374

Marcos-Filho, J., M. A. Bennett, M. B. McDonald, A. F. Evans, E. M. Grassbaugh. 2006. Assessment of melon seed vigour by an automated computer imaging system compared to traditional procedures. Seed Science and Technology. 34: 485-497.

Matthews, S. (1980). Controlled deterioration: A new vigour test for crop seeds. p.647-661. In: Seed Production (ed. P. D. Hebblethwaite), Butterworths, London, UK.

Matthews, S. 1993. Ageing tests as a basis for the evaluation of seed quality. Acta Horticulturae. 362, 251262.

Matthews, S., W. T. Bradnock. 1967. The detection of seed samples of wrinkle-seeded peas (*Pisum sativum* L.) of potentially low planting value. Proceedings of the International Seed Testing Association. 32: 553-563.

McCormac, A. C., P. D. Keffe, R. S. Draper. 1990. Automated vigour testing of field vegetables using image analysis. Seed Science and Technology. 18: 103-112.

McDonald, M. B. 1993. The history of seed vigor testing. Journal of Seed Technology, 17 (2): 93-100.

McDonald, M.B. 1999. Seed deterioration: physiology, repair and assessment. Seed Science and Technology. 27: 177-237.

McDonald, M., 2001. Standardization of Seed Vigor Tests. In: Seeds: Trade, Production and Technolog. Ohio State University. 200-208.

McDonald, M. B., B. R. Phannendranath. 1978. A modified accelerated aging vigor test procedure. Journal of Seed Technology. 3: 27-37.

Moore, R.P. 1969. History supporting tetrazolium seed testing. Proceedings of the International Seed Testing Association 32: 233-242.

Ozoban M., I. Demir. 2002. Longevity of pepper *(Capsicum annuum L.)* and watermelon *(Citrullus lanatus Duch.)* seeds in relation to seed moisture and storage temperature. Indian Journal of Agricultural Sciences. 72 (10): 589-593.

Panobianco, M., J. Marcos-Filho. 2001. Evaluation of the physiological potencial of tomato seeds by germination and vigor test. Seed Technology, 23: 151-161.

Panayotov, N., A. Aladzhadjiyan. 1999. Electrical and optical properties of pepper seeds through different periods of their development. Crop Science. 36 (9): 485 – 489 (Bg).

Panayotov, N. 2010 a. Viability and vigour of pepper seeds in period of their ageing. Scientific Works of Agricultural University-Plovdiv, LV (1): 347-352.

Panayotov, N. 2010 b. Heterogeneity of carrot seed in depends on their position on the mother plant. Folia Horticulture, 22 (1): 25-30.

Panayotov, N. 2013. Initial vegetative productivity of seeds - a method for determining their vigor. Assessment of vigor of pepper seeds. Plant Sciences. 4-5: 52-57 (Bg).

Panayotov, N. 2014. Assessment of vitality potential and storability of pepper seeds by the method of accelerated ageing. Bulgarian Journal of Agricultural Science. 20 (1): 133-139.

Panayotov, N. 2015, Seed science and factors of seed production of the vegetable seeds. Publishing house of the Agricultural University-Plovdiv, Bulgaria, 281 pages. (Bg).

Panayotov, N. 2016. Vigour, vigour tests and sowing potential of vegetable seeds. Agricultural science, VIII (20): 7-20.

Panayotov, N. 2023. Study on the storability of cape gooseberry (*Physalis peruviana* L.). Proceeding of V Agribalkan Congress, 20-23 September, 2023, Edirne Turkey, 593-606.

Peñaloza, P., R.-R. Gerardo, M. B. McDonald, M. A. Bennett. 2005. Lettuce (*Lactuca sativa* L.) seed quality evaluation using seed physical attributes, saturated salt accelerated aging and the seed vigour imaging system. Electronic Journal of Biotechnology. 8 (3): 299-307

Piana, Z., M. A. A. Tillmann, K. Minami. 1995. Evaluation of physiological quality of onion seeds and its relationship with production of vigorous seedlings. Rev. Bras. de Sementes. 2: 149-153.

Pollock, B. M., E. E. Roos. 1972. Seed and seedling vigor. In: Koslowski, T.T., ed. Seed biology. Academic Press, New York, NY, USA. 1: 314-388.

Powell, A.A. 1995. The controlled deterioration test. In: van der Venter, H.A., ed. Seed Vigour Testing Seminar. The International Seed Testing Association, Kopenhagen, Denmark. 73-87.

Powell, A. A., S. Matthews, 1981. Evaluation of controlled deterioration, a new vigour test for small seeded vegetables. Seed Science and Technology. 9: 633–640.

Powell, A. A., S. Matthews. 1984a. Prediction of the storage potential of onion under commercial storage conditions. Seed Science and Technology: 12, 641–647.

Powell, A. A., S. Matthews. 1984b. Application of the controlled deterioration test to detect seed lots of Brussels sprouts with a low potential for storage under commercial conditions. Seed Science and Technology. 12: 649–657.

Priestley, D. A. 1986. Seed Aging: Implications for Seed Storage and Persistence in the Soil. Comstock, Ithaca, NY, USA. 123 pages.

Priestley, J. T. 1958. Relation of protoplast permeability to cotton seed viability and predisposition to seedling disease. Plant Disease Reporter. 42: 852.

Prusinski J., M. Borovska. 1993. Application of electroconductivity test in vigor evaluation of mechanically damaged pulse crop seeds. Biuletyn Institutu Hodowly i Aklimatizacii Roslin. 186:133-144.

Ratajczak, K., K. W. Duczmai. 1991. Seed vigour estimation by thre conductometrical test. Biuletyn Institutu Hodowly i Aklimatizacii Roslin. 180: 181-188.

Report. 2024. Vegetable seeds global market. The business research company. (https://www.thebusinessresearchcompany.com/report/vegetable-seeds-global-market-report)

Rodo, A. B, J. Marcos-Filho. 2003. Accelerated aging and controlled deterioration for the determination of the physiological potential of onion seeds. Scientia Agricola. 60 (3): 465-469.

Sako, Y., A. Hoffmaster, K. Fujimura, M.B. McDonald, M.A. Bennett. 2002. Applications of computers in seed technology. In: McDonald and S. Contreras (Edts.) Proceedings International Seed

Seminar: Trade, Production and Technology. 172- 187.

Sako, Y., M. B. McDonald, K. Fujimura, A. F. Evans, M. A. Bennett. 2001. A system of automated seed vigour assessment. Seed Science and Technology. 29: 625-636.

Sales, T. S., V. C. de Andrade Júnior, Al. M. Azevedo, M. A.V. Santos, S. G. F. de Melo, M. C. Nery. 2022. Tetrazolium test to assess the viability of kale seeds. Journal of Seed Science. 44: 1-9.

Shoham J. 2024. Global Vegetable Seed Markets 2024. Vegetable demand drives global seeds growth. Consulting Analyst, Crop Science. S&P Global Commodity Insights Contributor. 1-33

Soltani, A., T. A. Lestander, M. Tigabu, P. C. Oden. 2003. Prediction of viability of oriental beechnuts, Fagus orientalis, using near infrared spectroscopy and partial least squares regression. Journal of near infrared spectroscopy. 11 (5): 357-364.

Stahl, C. 1931. Comparative experiments between the laboratory and the field germination of seed. Proceedings of the International Seed Testing Association. 15-17: 75-143.

Svetleva, D. and N. Panayotov, 2014. Report of Project. In: Scientific Collective: Complex morphometric, physiological, biochemical and molecular assessment of drought tolerance in

Bulgarian bean genotypes. Project DDVU-02/87. National Found “Scientific Research” 2011-2013.

Stahl, C. 1931. Comparative experiments between the laboratory and the field germination of seed. Proceedings of the International Seed Testing Association. 15-17: 75-143.

Tatum, L.A., M. S. Zuber. 1943. Germination of maize under adverse conditions. Journal of the American Society of Agronomy. 35: 48-59.

TeKrony, D.M. 1983. Seed vigor testing. Journal of Seed Technology. 8: 55-60.

Torres S. B. 1996. Physiologycal quality of pepper (*Capsicum annuum* L.) seeds measured by the water stress test. Revista Brasileira de Sementes. 2: 246-250.

Welbaum, G. E. 2024. Vegetable Seeds and Their Global Importance. CABI 200 Portland Street, Boston, USA

Welington, P. 1970. Handbook for seedling evaluation. Proceeding of the International Seed Testing Association, Zurich, Switzerland. 35(2): 100-170.

Wilson, D. O., M. B. McDonald. 1986. The lipid peroxidation model of seed ageing. Seed Science and Technology. 14: 269- 300.

Woodstock, L. W. 1965. Seed vigor. Seed World. 97: 6.

Woodstock, L. W. 1973. Physiological and biochemical tests for seed vigor. Seed Science and Technology. 1: 127-157.

Zhang, S., H. Zeng, W. Ji, K. Yi, S. Yang, P. Mao , Z. Wang, H. Yu, M. Li, 2022. Non-Destructive Testing of Alfalfa Seed Vigor Based on Multispectral Imaging Technology. Sensors. 22: 27-60.

INVESTIGATION OF ULTRASOUND TREATMENT ON THE VITALITY STATUS OF SEEDS FROM DIFFERENT SPECIES OF GENIUS ZINNIA ELEGANS JACK.

Valentin Panchev[1]

1Agricultural University - Plovdiv, 12, Mendeleev Blvd., 4000, Bulgaria

Corresponding author e-mail: valentin_panchev@abv.bg

ABSTRACT

In the present the effect of ultrasound on the sowing parameters of seeds from Zinnia elegans Jack. was studied. The experiments were carried out in the Department of Horticulture at the Agricultural University-Plovdiv, Bulgaria with three different varieties of this species. The seeds were treated with ultrasound for 2, 4, 6 and 8 minutes. The seeds were placed for germination according to the prescription of ISTA Rules. The following indexes were investigated: germination energy (first count) germination (final count), length of embryo root and hypocotyls as well as fresh weight of seedlings were observed. The mean germination time, uniformity of germination, and time of 50% germinated seeds were calculated. The highest increase of germination energy and germination was registered in variants 2- and 4-minute ultrasound. The differences with controls were between 1.0% and 25%. The highest correlation coefficients between the length of embryo root and hypocotyls were established. The regression analysis performed between germination and periods for sounding was registered and shows that there are polynomial dependencies with high determination coefficients.

Keywords seeds, energy of germination, germination, mean germination time, seedlings

INTRODUCTION

Zinnia is a widely used garden plant, both for landscaping in parks and gardens and for cut flowers. One of the difficulties encountered in its cultivation is related to its low sowing qualities and poor seed germination (Thamayanthi et al., 2011; Zamiran et al, 2013). Similar conclusions were formulated by Afzal et al. (2016), pointing out that from an agronomic point of view, one of the promising ways to overcome these disadvantages in zinnia seeds is their pre-sowing treatment.

Studies have been conducted to treat seeds of this crop with various agents. Ahmad et al. (2017) recommends the chemical treatment of zinnia seeds. Treatment with aqueous solutions of various compounds causes an improvement in sowing qualities. The best results were reported for seed treatment with calcium and potassium chloride, especially on germination and on the time to germination of 50% of the seeds.

According to Szopińska and Dorna (2023), the application of microwave irradiation to zinnia seeds can be used as an alternative to standard chemical seed treatment. In their experiments with zinnia seeds, they found that the strongest improvement in seed status was obtained with microwaves with an output power of 650 and 750 W. Rifna et al. (2019) point out that the use of ultrasound has a very positive effect on improving germination processes. Treatment of zinnia seeds with four different magnetic strengths of 15, 100, 400 and 800 μT showed that the best germination and emergence was found at 400 μT (Zamiran et al, 2013). Afzal et al. (2016) also found a high efficiency of physical treatment of seeds of this species with a magnetic field. The most significant increase in germination and seedling growth was obtained using 100 mT for 15 minutes.

Regarding soil contamination, Thamayanthi et al. (2011) reported that inhibition of zinnia seed and plant development was observed in the presence of cadmium. Rasool et al. (2020) investigated different ways of treating zinnia seeds. Hydro-priming with 3% moringa leaf extract significantly increases germination, accelerates sprouting and increases the length of the embryonic root. Treatment with magnetically treated water also showed a stimulating effect on the germination of 50% of the seeds, germination and plant development. The effect of combining these two methods of processing is much more significant, as the processes of germination and plant development, as well as yield characteristics are greatly improved.

To increase seed viability, Yaldagard et al. (2008) recommend using ultrasound. Khmelev and Popova (1997) point out that with such processing the seeds should not be more than 30% of the volume of water, and the duration of exposure is especially important. The importance of sonication time is also emphasized by Goussous et. al. (2010). Studies on the application of ultrasound for the treatment of zinnia seeds are very limited or almost non-existent, which determined the purpose of this study. The main aim of the present study was to determine the effect of sonication of zinnia seeds on their sowing qualities.

MATERIAL AND METHODS

The experiments were carried out in the scientific laboratory of the Department of Horticulture at the Agricultural University-Plovdiv, Bulgaria with three zinnia varieties Giant bright red, Giant white and Giant purple. Seeds were sonicated for 2-, 4-, 6-, and 8-minute. Untreated seeds were used as controls. The treatment was carried out with an Ultrasonic bath Nahita, 620-1 of the company Auxilab, S.L., Spain with parameters, frequency 40 KHz and volume 0.6 L.

For the germination test, seeds were placed in 10 cm Petri dishes lined with Watman 1 filter paper moistened with 5 ml of water. Experiments were carried out in four replicates with 100 seeds each, following the ISTA (2013) requirements.

Germinating energy (first cont|) and germination (final count) were recorded, and at the moment of germination determination, the length of the embryo root and hypocotyl on 15 seedlings from each replicate was measured. The fresh weight of a seedling was determined by measuring all the seedlings and dividing by their total number. Mean germination time (MGT) and uniformity of germination were calculated (described by Panayotov, 2015). The time for germination of 50% of the seeds was found by the equation of Farooq (2005). The correlation dependences between the fresh mass of the seedling and the length of the embryo root and the hypocotyl were determined. The regression relationship between the duration of ultrasound treatment and germination was established and the results were processed to ANOVA (Fowel and Cohen, 1992)

RESULTS AND DISCUSSION

In Table 1 the data on germination energy and germination of zinnia seeds after sonication is presented. The germination energy of the control seeds, depending on the variety, ranges from 14.3% for Giant white to 16.0% for Giant bright red. As a result of the action of the ultrasound, it increases to the variant of 4 minutes in all three varieties. The data between the three varieties are close, with the highest values of 21.3% reported for Giant bright red. The increase is between 5 and 7%. Stronger suppression was found after 6 and 8 minutes of exposure, except for Giant purple seeds. The reduction is in the range of 4-5%. Germination energy indicates the amount of earlier germinated seeds that have better viability (Panayotov 2015). In this sense, it can be seen that in the zinnia these seeds are relatively few.

For a more complete assessment of seed status, germination was used (Black et al., 2006). A similar trend as for sprouting energy is observed for this indicator, but at the same time, there is also a varietal reaction. The largest increase for Giant bright red with 14.66% and for Giant purple with 25.33% was found at 4 minutes of sonification. In the case of the Giant white variety, maximum values were registered at a lower duration of 2 minutes, and the increase compared to the control was 9.33%. At 6 and 8 minutes, germination slightly decreased, more pronounced for Giant purple, followed by Giant bright red.

The effect of ultrasound is debatable. One of the most widespread opinions is that during seed sonication, as a result of the waves, the pores of the seed coat are enlarged (López-Ribera and Vicient, 2017; Aladjadjiyan, 2007). In this way, the uptake of water and oxygen is accelerated, which has a positive effect on germination (Nogueira et al., 2024). This researcher also points out that this processing has a great influence on biochemical and molecular processes, especially on the breakdown of starch and activation of the enzyme α-amylases in the endosperm.

Chen et al. (2023) also emphasized the presence of cracks on the seed coat, which causes accelerated water absorption and its immobilization in the seed. In addition, the presence of changes in the metabolism of the seed, such as an increase in protease, amylase, lipase and peroxidase activity and especially phenylalanine ammonia lyase, as well as the accumulation of phenols and an increase in antioxidant activity, is also discussed. It can be assumed that the reported stimulation of zinnia seed vigor is related to the occurrence of these changes. The data for germination are statistically proven.

Table 1. Viability parameters of zinnia seed after treatment with ultrasound

Variants	Germination energy (%)			Germination (%)		
	Giant bright red	Giant white	Giant purple	Giant bright red	Giant white	Giant purple
Control	16.0	14.3	15.3	80.0	80.0	69,33
2 min.	19.7	18.3	19.3	93,33	89,33	86,66
4 min.	21.3	21.0	20.0	94,66	82,66	94,66
6 min.	17.7	16.0	20.0	92.0	82,66	92.0
8 min.	17.7	16.3	20.0	89,33	80.0	88.0
LSD p= 0.05%	3,3	2,0	4,1	8,2	7,5	11,2

The regression relationships between exposure and germination (Figure 1) confirm the indicated changes in germination, the dependence is of a polynomial type with high coefficients of determination for the varieties Giant bright red and Giant purple, respectively R^2= 0.89 and R^2= 0.97, and for Giant white the coefficient is average with R^2=0.44. These coefficients of determination show that in 44 to 97% of cases, sonication of zinnia seeds will produce the indicated trend. Using these coefficients, the influence of the factorial variable on the variation of the resulting variable can be determined.

Using the germination time of 50% of the seeds, a more complete assessment of their qualities is obtained (Trayanov 2021). The values of this indicator for zinnia seeds vary between 2 and 4 days (Table 2). The shortest period to reach 50% germination was observed with sonication of 4 minutes. The difference compared to untreated seeds, depending on the variety, is 2 days for Giant white and Giant purple and 1.33 days for Giant bright red. Weak inhibition was observed for the 6 and 8-minute variants, with an increase in this period of approximately one day. It can be pointed out that the variety's responsibility, in contrast to germination, is weaker or almost absent here. The lowest results are reported for the 8-minute option.

Improvement of this parameter in the pre-sowing treatment of zinnia seeds was reported also by Rasool et al. (2020) and Afzal et al. (2016).

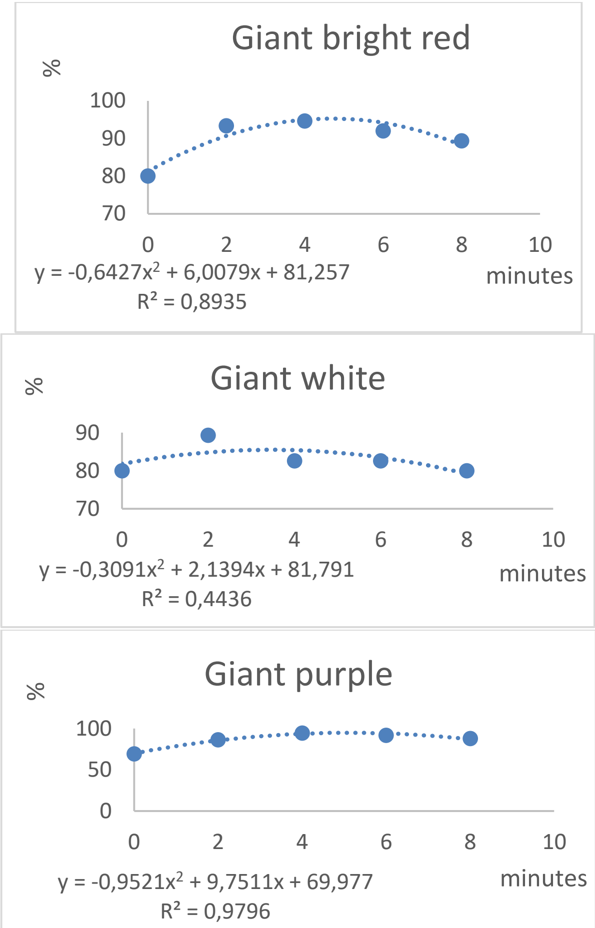


Figure 1 . Regression dependence of germination and the duration of ultrasound treatment of zinnia seeds

Table 2. $T_{50\%}$ germination of different zinnia varieties after ultrasound treatment (days)

Variants	Giant bright red	Giant white	Giant purple
Control	3,33	4.0	4.0
2 min.	2,66	2,66	3,33
4 min.	2.0	2.0	2.0
6 min.	2,33	2,66	3.0
8 min.	3.0	2,66	3.5
LSD p= 0.05%	1,2	1,4	1,6

To more fully trace the impact of ultrasound, it is appropriate to determine the mean germination time (Table 3). Seed treatment reduces the time required for seed germination in almost all exposures and cultivars. In the shortest time, the seeds germinated at 4 minutes of sounding for the variety Giant bright red, followed by Giant purple, and the differences compared to the control were 0.96 and 1.53 days, respectively. After this variant, the values decrease but are still higher than those of untreated seeds except for the Giant white variety at 6 and 8 minutes.

The effect of sounding is also established on uniformity, which indicates the simultaneity of germination, which is a prerequisite for easier seed germination. The seeds treated for 4 minutes showed the greatest uniformity of germination in all three varieties. The largest difference compared to the control was found in Giant purple, almost 7%, followed by the seeds of the other two varieties, for which the increase was 3.5%. Increasing the period of sounding leads to weak inhibition especially at 8 minutes, except the variety Giant purple. In Giant white, suppression was also observed at 6 minutes of ultrasound.

Table 3. Sowing indexes of zinnia seed after treatment with ultrasound

Variants	Mean germination time (days)			Uniformity of germination (%)		
	Giant bright red	Giant white	Giant purple	Giant bright red	Giant white	Giant purple
Control	3,35	3,37	4,09	14.0	14.7	11.0
2 min.	3,12	2,94	3,28	14.8	15.3	14.3
4 min.	2,41	2,80	2,56	17.4	18.3	18.1
6 min.	3,26	3,48	3,02	16.6	13.3	15.3
8 min.	3,10	3,41	3,49	13.3	13.9	14.5
LSD p= 0.05%	2,0	1,9	1,2	2,2	2,6	3,0

The morphological features of the sprouts describe their development very well and complete the picture of the vital status of the seeds and the possibility of normal formation of young plants. The data are shown in Table 4. Stronger embryo root developed after sounding the seeds of Giant bright red (4.97 cm) for 4 minutes, while for the other two varieties, it was at 2 minutes, respectively 4.41 cm for Giant white and 4.51 cm for Giant purple. The increase in its length over the control was between 20.91% (Giant purple) and 87.8% (Giant bright red). The two higher exposures caused inhibition except for the Giant bright red variety.

Hypocotyl length was greatest after exposure to 2 minutes of ultrasound. Maximum values were measured for Giant purple, which is 52.68% above the control, followed by Giant white with an increase of 14.8%. All the tested durations of ultrasound treatment promote stronger development of the hypocotyl, compared to untreated seeds, except the variety Giant white, in which inhibition is established as early as 4 minutes. Both embryo root and hypocotyledon length decreased uniformly after 2 min of exposure.

The fresh weight of a seedling (Table 5) is an important indicator of its morphological development. It is directly related to vigor, which also determines the possibility of further development of young plants (Panayotov, 2016). In all exposures, the application of ultrasound increased fresh weight. The highest values for all three tested cultivars were recorded for processing for 4 minutes. Giant white seeds developed the largest weight – 0.95 mg or 86.26% more than the control. In the other two varieties, the weight was 0.64 mg and 0.68 mg for Giant bright red and Giant purple, respectively, and the increase compared to untreated seeds was approximately between 25 and 30%. At 6 and 8 minutes, a stimulating effect is also found, but weaker than for the 4-minute option. As the seed sonication time increased after 4 min, a uniform decrease was observed. Improvement of the morphological seedling development after the pre-sowing treatment of the zinnia seed establishes also Afzal et al. (2016).

Correlational dependences were established between the length of the embryo root and the hypocotyl to the fresh weight of the seedling. A stronger relationship is found for hypocotyl length, the correlation being strong and positive with a coefficient of r=0.67 to r=0.96. Embryo root length has a weaker influence, where the correlation is also positive but weak to moderate.

Table 4. Morphological characteristics of of zinnia seed after treatment with ultrasound

Variants	Length of embryo root (cm)			Length of hypocotyl (cm)		
	Giant bright red	Giant white	Giant purple	Giant bright red	Giant white	Giant purple
Control	2,93	3,32	3,73	2,20	4,22	3,91
2 min.	4,20	4,41	4,51	3,27	4,83	5,97
4 min.	4,97	3,47	4,37	3,27	3,90	5,63
6 min.	4,30	2,60	3,47	2,43	3,80	4,50
8 min.	4,70	1,97	3,07	2,42	3,17	4,42
LSD p= 0.05%	2,6	2,8	1,5	1,6	1,8	2,2

Table 5. Fresh weight of one sprout of different zinnia varieties (mg)

Variants	Giant bright red	Giant white	Giant purple
Control	0,51	0,51	0,46
2 min.	0,51	0,91	0,58
4 min.	0,64	0,95	0,68
6 min.	0,61	0,62	0,61
8 min.	0,58	0,62	0,56
r*	0,21	0,57	0,53
r**	0,96	0,75	0,67
LSD p= 0.05%	0,3	1,4	2,0

r* - correlation between fresh weight and length of embryo root,
r** - correlation between fresh weight and length of hypocotyl

CONCLUSIONS

Ultrasound treatment of zinnia seeds has a strong influence on their sowing qualities. The highest germination energy and germination were found after the application of exposure of 2 and 4 minutes. A polynomial relationship with high coefficients of determination was established between processing time and germination.

The strongest positive effect on the germination time of 50% of the seeds, as well as the mean germination time and uniformity, is also found at 4 minutes of ultrasound.

Seedlings from seeds treated with ultrasound have improved vegetative development, with the length of the embryo root and the fresh weight of one seedling being the highest when applied for 4 minutes, and for the hypocotyl-exposed for 2 minutes.

To improve the sowing qualities of zinnia seeds for practice, it is recommended to apply ultrasound treatment for 4 minutes.

REFERENCES

Afzal, I., K. Abbasi, Iqbal, Younis A, A. Bakhtavar, & H. U. Rehman. 2016. Enhancement of zinnia seed germination and seedling emergence through magnetic seed stimulation. *Acta Scientiarum Polonorum Hortorum Cultus*, *15*(5): 73-184.

Ahmad, I., A. M. Saleem, G. K. MustafaZiaf, I. Afzal, & M. Qasim. 2017. Seed halopriming enhances germination performance and seedling vigor of gerbera jamesonii and zinnia elegans. *Sarhad Journal of Agriculture*, *33*(2), 199-205.

Aladjadjiyan, A. 2007. The use of physical methods for plant growing stimulation in Bulgaria. J*ournal Central European Agriculture*, 8(3), 369-380.

Black, M., J. D. Bewley, P. Halmer. 2006. The encyclopedia of seed science, technology and uses. Cromwell Press, Trowbridge, UK. 828 pages.

Chen, J., F. Shao, C. J. Igbokwe, Y. Duan, M. Cai, H. Ma, & H. Zhang. 2023. Ultrasound treatments improve germinability of soybean seeds: The key role of working frequency. *Ultrasonics Sonochemistry*, *96*, 106434.

Farooq, M., S. M. A. Basra, N. Ahmad, K. Hafeez. 2005. Thermal Hardening: A New Seed Vigor Enhancement Tool in Rice. Journal of Integrative Plant Biology. 47 (2): 187−193.

Fowel, J., L.Cohen. 1992. Practicle statistics for field biology. John Wiley & Sons, New York. 223 pages .

Goussous, S., N. Samarah, A. Alqudah and M. Othman. 2010. Enhancing seed germination of four crop species using an ultrasonic technique, *Experimental Agriculture,* 46(2), 231–242.

ISTA 2013. International Rules for Seed Testing. International Seed Testing Association. Bassersdorf, CH-Switzerland. 265 pages.

Khmelev, V. and O. Popova. 1997. "Multifunctional ultrasonic devices and their application in small production, agriculture and at home". *Barnaul, AltSTU,* 112-116. (Ru.)

López-Ribera, I., & C. M. Vicient. 2017. Use of ultrasonication to increase germination rates of Arabidopsis seeds. *Plant Methods*, *13*(1), 1-6.

Panayotov N. 2015. *Seed science and factors of seed production of the vegetable crops.* Academic press of Agricultural University-Plovdiv. 281.

Rasool, S., I. Ahmad, K. Ziaf, I. Afzal, M. A. Khan, M. A. Sajjad, & M. Z. Ali. 2020. Seed Conditioning Effects on Germination Performance, Seedling Vigor and Flower Production of Zinnia elegans. *Sarhad Journal of Agriculture*, *36*(4).

Rifna, E. J., K. R. Ramanan, & R. Mahendran. 2019. Emerging technology applications for improving seed germination. *Trends in Food Science & Technology*, *86*, 95-108.

Szopińska, D., H. Dorna. 2023. Enhancing Zinnia (Zinnia elegans Jacq.) Seed Quality through Microwaves Application. *Agronomy*, *13*(5), 1241.

Thamayanthi, D., P. S. Sharavanan, & M. Vijayaragavan. 2011. Effect of cadmium on seed germination, growth and pigments content of Zinnia plant. *Current Botany*, *2*(8). ISSN: 2220-4822.

Traynov, Al. 2021. Productivity and quality of carrot seeds through optimization of the nutrient regime in their seed production. PhD thesis. Agrivultural Univeristy-Plovdiv, 218.

Yaldagard, M., S. Mortazavi and F. Tabatabaie. 2008. Application of ultrasonic waves as a priming technique for accelerating and enhancing the germination of barley seed: optimization of method by the taguchi approach. *J. Inst. Brew*., 114(1), 14-21.

Zamiran, A., V. R. Saffari, & M. R. Maleki. 2013. Seed Germination Enhancement of Zinnia (Zinnia elegans) Using Electromagnetic Field. *Journal of Ornamental & Horticultural Plants*, *3*(3).

CHERRY FRUIT PHYSICAL, ORGANOLEPTIC AND PHYTOCHEMICAL CHARACTERISTICS OF TWELVE CULTIVARS GROWN IN ARNISSA, CENTRAL MACEDONIA, GREECE

Asimina-Georgia Karida[1], Panagiota Kosmadaki[1], Chrysa Kotsi[1], Dionisios Gasparatos[2], Petros A. Roussos[1]

[1] Agricultural University of Athens, Laboratory of Pomology, Iera Odos 75, Athens 118 55, Greece
[2] Agricultural University of Athens, Laboratory of Soil Science and Agricultural Chemistry, Iera Odos 75, Athens 118 55, Greece
Corresponding author e-mail: roussosp@aua.gr

ABSTRACT

Cherry fruit is a drupe which is highly appreciated by consumers. Many cultivars have been developed around the world, based mainly on physical characteristics, such as size and color. Not all cultivars are suitable for a specific region and trials should be made before deciding which cultivar is best suited for this specific pedoclimatic conditions. Tweleve cultivars grown in the same are, Arnissa, Western Greece, were assessed for their physical, organoleptic and phytochemical characteristics. Fruits were provided by the Co-operative of Arnissa and were harvested at the commercial maturity of each cultivar. Cultivars assessed were “Samba”, “Kordia”, “Lapins”, “Germesdorfer”, “Grace Star”, “Durone Nero III”, “Crystallina”, “Regina”, “Skeena” and local ones such as “Opsimi”, “Tragana Edessas” and “Evlogimeno” while samples of “Regina” (REGA) from a specific location in the area were also assessed. Physical characteristics measured were fruit weight and size (diameter (wide and narrow), length and flesh weight, as well as skin color, expressed as Hue angle and Chroma indexes). Furthermore, total soluble solids, pH and titratable acidity were assessed in the pulp as well as total phenols, total flavanols, total flavonoids and antioxidant capacity, based on Diphenyl picryl hydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays. Results indicated that there were significant among cultivars regarding the measured parameters. Principal component analysis indicated that “Tragana Edessas” as well as “Durone Nero III” were distinguished from the other cultivars based mainly on their phenol content and antioxidant capacity. The highest fruit weight was determined in “Durone Nero III” and “Crystallina” fruits, while “Durone Nero III” also presented the highest total soluble solids, in contrast to “Evlogimeno” and “Germesdorfer” which presented the lowest. These significant differences among cultivars should be taken into account, along with the maturity period and tree load when someone needs to select the proper cultivar or cultivars for specific pedoclimatic conditions.

Keywords: antioxidant capacity, cherry, organoleptic characteristics, phenolic compounds

INTRODUCTION

Sweet cherry is a highly valued premium food product with high market prices, highly appreciated by consumers and of high commercial importance in many countries in the world (Ricardo-Rodrigues et al. 2022). Among the factors determining consumer’s appreciation are the fruit size and color and sweetness (Serrano et al. 2009). The total soluble solids (TSS)/titratable acidity (TA) ratio has an important impact on the perception of sweetness and flavour, and therefore on the overall acceptance by consumers (Crisosto et al. 2003). Nowdays people seek not only pleasent taste in a fruit but also functional properties which may reduce incidence of degenerative diseases due to their antioxidant potential (Kris-Etherton et al. 2002). Substances that are responsible for the antioxidant properties of the fruits are mainly ascorbic acid, polyphenols, tocopherols, carotenoids etc. Cherry fruit is rich in anthocyanins, which, as part of the polyphenol group, play an important role in the antioxidant property of the fruits as well as in fruit color.

Nonetheless, the organoleptic, nutritive and functional properties of the fruit differ among cultivars (Serrano et al. 2009) and depend on many factors such as vary the maturity stage of the fruit, the cultural practices, and environmental factors (Aglar et al. 2023).

The aim of the present research was to assess the physical, organoleptic and functional properties of the fruit of several sweet cherry cultivars, cultivated under the same pedoclimatic conditions and similar cultural management (as possible as it can be), all harvested at full maturity stage, ripe ready to eat.

MATERIAL AND METHOD

The trial was conducted at the Agricultural Co-operation of Arnissa, Central Macedonia, Greece (Figure 1). The fields were located near the lake Vegoritida, which offers mild weather conditions, suitable for cherry growing.

Twelve cherry cultivars were selected (Table 1), located in seven different farms, cultivated by four different farmers. The cultivation management applied was similar to all farms, based on information received by the farmers. Nonetheless, “Regina” fruits were selected by a second farm too (REGA) in order to assess if the specific pedoclimate conditions and cultural management of the farmer may play a significant role in fruit’s properties. Fruits were harvested at ready to eat maturity stage by the farmers and send to the laboratory within the day.

Table 1. List of the cultivars assayed.

Crystallina (CRY)	Grace Star (GS)	Regina (REG)
Durone Nero III (DN3)	Kordia (KOR)	Skeena (SK)
Evlogimena (EVL)	Lapins (LAP)	Tragana Edessas (TRA)
Germesdorfer (GRS)	Opsimi (OPS)	Samba (SB)

Malformed fruits or fruits sowing any symptoms of wound were discarded and the rest of the fruits were assessed for their physical, organoleptic and functional properties.

Fruit size (diameter – wide and narrow - and length) was determined with a digital caliper, fruit weight with an electronic balance and skin color with a Minolta Chromameter.

Figure 1. Location of Arnissa village, where the trial took place.

Fruits were de-stoned and the flesh was then homogenized with a home-homogenizer and the pulp produced was put in falcons tubes and stored in the freezer till analysis, which were all were done according to Roussos et al. (2022). The total soluble solids (TSS) of the pulp were measured with a digital refractometer, pH with a pH-meter and total acidity (TA) with titration, on the de-freezed pulp. Total phenols, total flavanols, total flavonoids, and the antioxidant capacity of the fruit (based on two assays, the Diphenyl Picryl Hydrazyl (DPPH) assay and the Ferric Reducing Antioxidant Power (FRAP) assay) were determined after pulp extraction with 100% methanol, twice in a water bath at 45^{o}C with periodical stirring, for 45 min each time. The extracts were centrifuged and the supernatant was used for the determination of the functional properties of the fruit as described by Roussos et al. (2022). The total phenols were expressed in mg gallic acid per g of fresh weight, the total flavanols in mg catechin per g of fresh weight, total flavonoids in mg catechin per g of fresh weight and antioxidant capacity in μmol Trolox per g of fresh weight, for both method assays.

The trial was conducted as a completely randomized design with three replications and the data were analyzed as a one-way Anova and the significant differences were determined based on Tukey HSD test at a=0.05. Principal component analysis also took place in order to describe cultivars relation using fewer variables.

RESULTS AND DISCUSSION

Fruit weight varied among cultivars, with the lowest being determined in TRA and the highest in CRY and DN3, reaching high values, higher than that reported for other cherry cultivars by Lanauskas et al. (2023). The same stood also for fruit size (diameters and length) as well as for the flesh weight. It is notable that Regina (REG and REGA) did not present significant differences, although they derived from different orchards, indicative that some traits of the cultivar are not influenced by cultivation management. Nonetheless, the color differed significantly, both between REG and REGA as well as among cultivars. The highest Hue angle was detected in SK fruits while the lowest in SB. The Chroma index was highest in OPS and lowest in KOR. Similar differences among cultivars have been detected by other researchers too, indicating that the genotype has an important influence on the physical traits of the fruit (Dever et al, 1996, Girard and Kopp, 1998. Lanauskas et al. 2023).

Table 1. Physical characteristics of the various cultivars assayed.

Cultivar	Weight (g)		DS (mm)		DC (mm)		L (mm)		Flesh weight (g)		Hue angle		Chroma	
CRY	11.42	a	23.41	abc	29.05	a	25.69	a	10.67	a	20.49	efg	16.39	bc
DN3	11.53	a	24.08	a	28.79	ab	24.56	abc	10.72	a	22.68	ef	13.03	cd
EVL	7.43	e	20.44	f	27.14	abc	21.21	e	6.85	e	37.86	bc	10.02	defg
GRS	9.09	cde	22.61	abcd	26.64	bcd	23.26	cd	8.44	bcde	41.05	b	9.99	defg
GS	8.41	de	20.47	ef	26.54	bcde	21.97	de	7.67	de	38.31	bc	7.63	efg
KOR	10.26	abc	22.26	bcde	26.49	bcde	25.36	a	9.67	ab	30.46	d	6.05	g
LAP	8.44	de	21.21	def	26.34	cde	22.27	de	7.82	cde	33.67	cd	7.86	efg
OPS	9.19	bcde	23.06	abc	26.06	cde	24.89	ab	8.57	bcde	24.80	e	29.27	a
REGA	10.89	abc	22.87	abcd	25.28	cdef	24.09	abc	10.14	ab	37.90	bc	10.73	def
REG	10.32	abc	23.92	ab	24.73	def	24.68	abc	9.54	abc	19.91	efg	20.28	b
SB	10.10	abcd	23.11	abc	24.19	ef	24.57	abc	9.55	abc	17.40	g	18.04	b
SK	9.96	abcd	22.07	cdef	23.28	f	23.47	bc	9.08	abc	46.88	a	6.83	fg
TRA	4.87	f	18.17	g	20.65	g	18.58	f	4.43	f	19.42	fg	11.79	cde

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

Significant differences among cultivars were also detected regarding organoleptic characteristics. The pH value was lowest in SK and highest in REG, which presented significant difference from REGA. pH values ranged from 2.24 to 2.80, lower than that described by Seradilla et al (2012) probably a result of different pedoclimatic conditions and of course genetic material. The TSS was highest in DN3 and lowest in OPS ranging from 13.30 to 25.17, similar to that reported by Dangi et al. (2024), Serrano et al. (2009) and Lanauskas et al. (2023). The titratable acidity ranged from 0.80 in TRA to 1.59 in SB, higher than that reported by Dangi et al. (2024) who also reported higher values of TSS:TA ratio but similar to that reported by Serrano et al. (2009) and Serradilla (2012). In our experiment this ratio ranged from 10.71 in OPS to 24.91 in TRA, indicative of the influence of the genetic material.

Table 2. Organoleptic characteristics of the various cultivars assayed.

Cultivar	pH		TSS (ºBrix)		TA (% malic aid)		TSS/TA	
CRY	2.38	cde	22.93	c	1.52	ab	15.11	bcd
DN3	2.58	abcd	25.17	a	1.12	de	22.65	a
EVL	2.48	bcde	18.27	i	1.19	cde	15.43	bcd
GRS	2.72	ab	18.13	i	1.10	de	16.66	bc
GS	2.65	abc	23.70	b	1.43	abc	16.61	bc
KOR	2.34	de	18.87	h	1.48	abc	12.84	cd
LAP	2.76	ab	17.50	j	1.30	abcd	13.61	cd
OPS	2.63	abcd	13.30	k	1.25	bcde	10.71	d
REGA	2.36	de	20.23	f	1.25	bcde	16.21	bcd
REG	2.80	a	19.17	g	0.99	ef	19.51	ab
SB	2.38	cde	22.53	d	1.59	a	14.22	bcd
SK	2.24	e	20.90	e	1.54	ab	13.55	cd
TRA	2.60	abcd	19.33	g	0.80	f	24.91	a

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

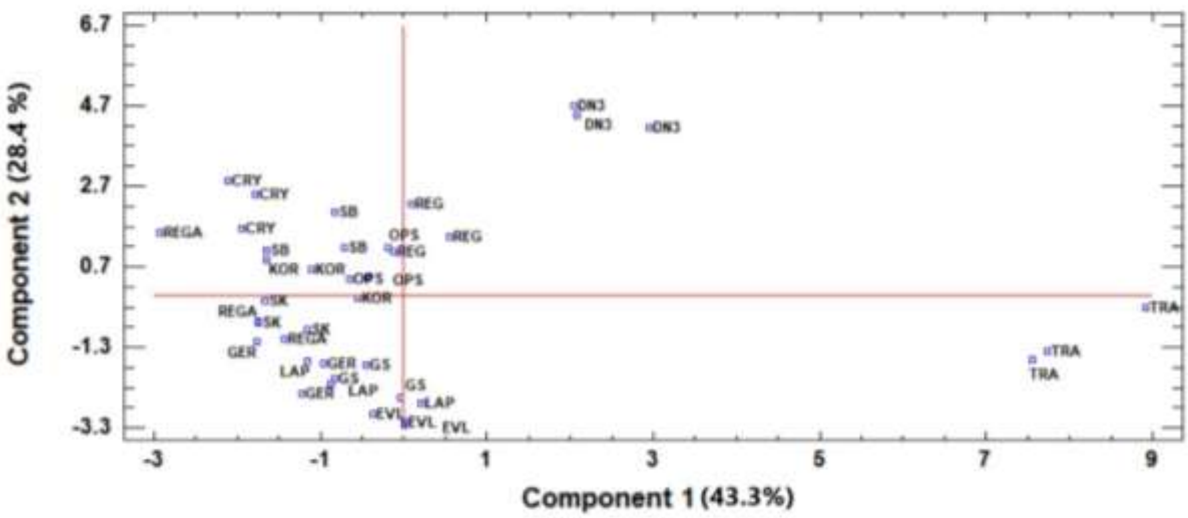
There were significant differences among cultivars concerning the functional properties of the fruit, as indicated in Table 3. The highest total phenols were determined in the fruit of TRA followed by those of DN3, while the lowest in GRS. Similarly, TRA presented the highest total flavanols and total flavonoids concentration while the lowest were determined in GRS. The total antioxidant capacity of the fruit according to the DPPH assay was highest in TRA and lowest in GRS which also presented lowest FRAP values. Differences in the total phenolic content and antioxidant capacity among cultivars have been found by other researchers too, indicative of the effect of the genetic material on the functional properties of the fruit (Serrano et al. 2009; Serradilla et al. 2012; Dangi et al. 2024). It must be noted though, that REG and REGA presented significant differences regarding total flavanols, total flavonoids, DPPH and FRAP activities, which clearly indicate that the cultivation management plays also a significant role, among other factors.

Table 3. Functional characteristics of the various cultivars assayed.

Cultivar	**Total phenols (mg GAE/g FW)**		**Flavanols (mg CAE /g FW)**		**Flavonoids (mg CAE /g FW)**		**DPPH (µmol Trolox /g FW)**		**FRAP (µmol Trolox /g FW)**	
CRY	0.72	bcd	0.039	efg	0.222	cde	3.21	cde	9.74	bcd
DN3	1.66	a	0.162	b	0.611	b	7.68	b	27.77	a
EVL	0.41	de	0.003	g	0.129	def	2.01	ef	4.77	def
GRS	0.33	e	0.002	g	0.091	f	1.66	ef	2.59	f
GS	0.53	bcd	0.010	fg	0.147	cdef	2.19	deg	5.35	cdef
KOR	0.85	b	0.059	cde	0.233	cde	3.96	c	11.49	b
LAP	0.47	cde	0.005	g	0.144	cdef	2.47	def	5.82	cdef
OPS	0.84	b	0.087	c	0.235	cde	4.27	c	9.64	bcd
REGA	0.48	cde	0.023	efg	0.127	ef	2.14	deg	3.58	ef
REG	0.74	bcd	0.083	cd	0.246	cd	4.41	c	10.35	bc
SB	0.78	bc	0.050	cde	0.247	c	3.55	cd	10.08	bc
SK	0.71	bcd	0.047	def	0.203	cdef	3.07	cdef	8.29	bcde
TRA	1.77	a	0.224	a	0.821	a	9.44	a	28.71	a

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

The principal component analysis (Figure 2) presented interesting results, as TRA was clearly separated from the other cultivars as well as DN3, while REG and REGA were also separated, indicating that the genetic material is not the only factor influencing fruit physical, organoleptic and functional properties.



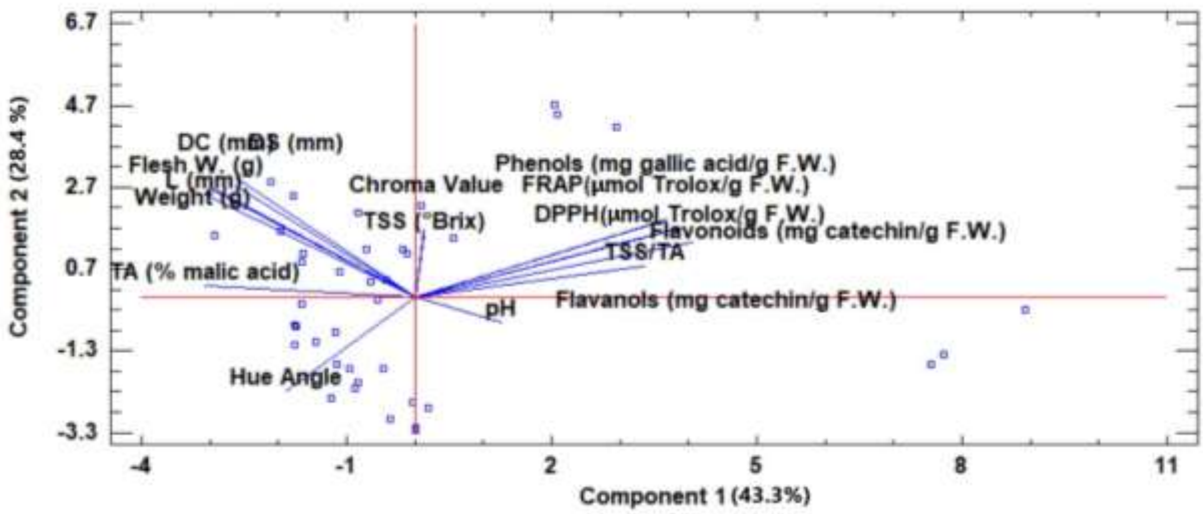


Figure 2. Scatterplot (above) and plot of components (below) of the principal component analysis taking into account all measured variables.

CONCLUSIONS

Based on the present study, it became clear that there are significant differences among cultivars regarding all three physical, organoleptic and functional properties. Unfortunately, the attractive appearance of a fruit does not always accompanied by the best functional properties, as it was clearly indicated by the data presented. It was also clear that the genetic material is not the only factor influencing the fruit traits studied here, as the same cultivar, deriving from two different orchards, presented both similar as well as different fruit characteristics.

REFERENCES

Aglar, E., O. Saracoglu, B. Ozturk, O. Karakaya, U. Ateş. 2023. The Influence of Fruit Size on Quality Attributes and Bioactive Compounds of Sweet Cherry Fruit. Erwerbs-Obstbau. 65(4): 701-707

Crisosto, C.H., G.M. Crisosto, P. Metheney. 2003. Consumer Acceptance of ‘Brooks’ and ‘Bing’cherries is Mainly Dependent on Fruit SSC and Visual Skin Color. Postharvest Biology and Technology, 28(1):159-167

Dangi, G., D. Singh, N. Chauhan, R.K. Dogra, P. Verma, A. Chauhan. 2024. Evaluating Genetic Diversity of Morpho-Physiological Traits in Sweet Cherry (*Prunus Avium* L.) Cultivars Using Multivariate Analysis. Genetic Resources and Crop Evolution. 1-36

Dever, M.C., R.A. MacDonald, M.A. Cliff, W.D. Lane. 1996. Sensory Evaluation of Sweet Cherry Cultivars. HortScience. 31(1): 150-153

Girard, B., T.G. Kopp. 1998. Physicochemical Characteristics of Selected Sweet Cherry Cultivars. Journal of Agricultural and Food Chemistry. 46(2): 471-476

Kris-Etherton, P.M., K.D. Hecker, A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert, A.E. Griel, T.D. Etherton. 2002. Bioactive Compounds in Foods: Their Role In The Prevention Of Cardiovascular Disease And Cancer. The American Journal of Medicine. 113(9): 71-88

Lanauskas, J., D. Kviklys, N. Uselis, V. Stanys. 2023. Performance of Sweet Cherry Cultivars and Advanced Selections on Gisela 5 Rootstock in Young Orchards. Plants, 12(3), p.614

Roussos, P.A., A. Triantafillidis, E. Kepolas, P. Peppas, A. Piou, M. Zoti, D. Gasparatos. 2022. Effects of Integrated and Organic Management on Strawberry (Cv. Camarosa) Plant Growth, Nutrition, Fruit Yield, Quality, Nutraceutical Characteristics, and Soil Fertility Status. Horticulture. 8(2): 184

Serradilla, M.J., A. Martín, S. Ruiz-Moyano, A. Hernández, M.López-Corrales, M. de Guía Córdoba. 2012. Physicochemical and Sensorial Characterisation of Four Sweet Cherry Cultivars Grown in Jerte Valley (Spain). Food Chemistry. 133(4): 1551-1559

Serrano, M., H. M. Díaz-Mula, P. J. Zapata, S. Castillo, F. Guillén, D. Martínez-Romero, J.M. Valverde, D. Valero. 2009. Maturity Stage at Harvest Determines the Fruit Quality and Antioxidant Potential After Storage of Sweet Cherry Cultivars. Journal of Agricultural and Food Chemistry. 57(8): 3240-3246

THE EFFECTS OF ATTAPOULGITE ALONE PLUS OLIVE MILL WASTE ON OLIVE YIELD, OLIVE OIL QUALITY, LEAF NUTRIENT STATUS AND SOIL PROPERTIES

Asimina-Georgia Karyda[1], Vicky Christopoulou[2], Dionisios Gasparatos[3], Petros A. Roussos[1]

[1] *Agricultural University of Athens, Laboratory of Pomology, Iera Odos 75, Athens 118 55, Greece*
[2] PUREOLEA Estate & Olive Mill, Agios Vlasis, Ypsilantis Viotia PC 32001, Greece
[3]*Agricultural University of Athens, Laboratory of Soil Science and Agricultural Chemistry, Iera Odos 75, Athens 118 55, Greece*
Corresponding author e-mail: roussosp@aua.gr

ABSTRACT

The olive oil industry is considered one of the most important sectors in agriculture, especially for the countries in the Mediterranean basin. One of the key challenges the sector faces is the recycling and use of olive mill wastes. The aim of this trial was to investigate how a mixture of a soil amendment with olive mill waste can be utilized in olive groves. Olive mill waste derived from a two-phase olive mill was mixed with attapulgite and applied as a soil amendment to mature, bearing olive trees of the cultivar "Megareitiki," grown under rainfed conditions. The application was made in early spring, and the two constituents were mixed in a 1:1 ratio. The mixture was applied at a rate of 8 kg per tree. Untreated trees served as controls, while a third treatment involved the soil application of attapulgite at a rate of 4 kg per tree. At harvest in early November, the length of annual shoot growth was measured, and the trees were individually harvested and their yield measured. A sample of approximately 1.5 kg of olive fruits was used for oil extraction in an Abenchor-type laboratory olive mill to determine oil percentage. The olive oil produced was assessed for acidity, peroxide number, UV absorbance (K values), polyphenols, and antioxidant capacity. At the same time, soil samples were taken from 0-30 cm and 30-60 cm depths to assess soil physicochemical properties, and leaf samples were taken to assess tree nutrient status. The treatments did not have any significant effect on shoot growth (ranging from 15.2 to 16.4 cm), yield, olive oil percentage, or oil quantity per tree. Olive oils produced under the different treatments were all classified as Extra Virgin Olive Oil based on the measured variables, with none of the treatments having a significant effect on them. The addition of attapulgite resulted in higher concentrations of N-NO_3, B, and Na in the soil (0-30 cm), while the mixture of olive mill waste and attapulgite resulted in higher P and Mn concentrations. At the depth of 30-60 cm, higher P concentrations were found in the control and attapulgite treatments, while higher Fe concentrations were found in the control. Attapulgite addition to the soil resulted in higher B and Mn, while the application of the mixture of olive mill waste plus attapulgite increased the concentration of N-NO3. In the leaves, the addition of the mixture resulted in a significant increase in P and Mg, with no other differences observed. It seems that olive mill waste can be used in a mixture with attapulgite to increase some minerals in the soil without affecting olive tree production or olive oil quality. However, a longer experimental period is required to fully understand the possible effects of this mixture on both soil properties and leaf mineral nutrient concentration, yield, and olive oil properties.

Keywords: nutrients, olive oil, phenolics, soil properties

INTRODUCTION

The olive tree is the iconic tree of Mediterranean basin, highly appreciated not only for the nutritional value of its product, but also for its tolerance against abiotic stress factors, such as salinity and water stress (Denaxa et al. 2020). Olive oil is considered as one of the basic ingredients of a healthy diet, and its value is worldwide recognized. During the olive oil extraction though a significant amount of wastes are produced. The olive mills that are nowadays operating to extract the olive oil from the fruit are categorized as two-way and three-way mills, based on the number of phases produced at the end of the oil extraction procedure. The two-way olive mills apart from the olive oil, produce also a fluid mixture of olive paste residues (consisted of olive pulp and stone as well as water and water soluble substances, among others). This waste is extemely difficult to handle without any environmental concerns. A lot of research has been focused on the exlpoitation of olive mill wastes (OMW), exploring various methods of making this waste a valueable product (Chatzistathis and Koutsos, 2017).

The aim of the present trial was to investigate the possibilty of using OMW in a mixture with attapulgite, in order to be able to use it as soil amendment in olive trees.

MATERIAL AND METHOD

OMW deriving from a two-phase olive mill was used in the present trial along with attapulgite (AGLEV SI 100, GeoHellas, Greece). The attapulgite was selected after an initial trial in various test plants in pots (spinach, strawberry, olive trees, lettuce, pelargonium) where zeolite and attapulgite were tested in various ratios with OMW and the growth of the plants as well as signs of toxicity symptoms were assayed (data not presented). The trial was conducted at the Pure Olea Estate and Olive mill olive groves in Viotia county central Greece (Figure 1). The OMW was mixed with attapulgite in a ratio 1:1 and the mixture was applied in mature, rainfed, productive olive trees cv "Megareitiki" at a dose rate of 8 Kg per tree in early spring. Attapulgite applied alone at a dose rate of 4 Kg per tree was the other treatment, apart from the control.

At harvest, each tree was separately harvested and the production was weighed. At the same time the length of new shoot growth was measured on at least ten shoots per tree. Then a sample of at least 1.5 Kg of olive fruits were transferred to the laboratory where they were processed in a laboratory olive mill (Callis Company, Athens, Greece) and the olive oil percentage was calculated. The paste was put in the mixer for 35 min under 26±2 ^{o}C and afterwards centrifuged for 3 min. The olive oil was collected and stored in 50 mL falcon-type tubes, filled to the top to minimize air presence till analyses. The olive oils were analyzed for free acid concentration (expressed as a percentage of oleic acid), peroxide value (expressed as meq. O_2/kg oil), and specific extinction values (K232 and K270) based on EU Commission Regulation N 2568/91/EEC (Commission 1991). Polyphenols were isolated through liquid-liquid extraction with methanol/water (80: 20 v/v) solution (Cecchi et al. 2018). The total phenols (TPHEN, as mg gallic acid equivalents Kg-1 oil), the total o-diphenols (oDs, as mg caffeic acid equivalents Kg-1 oil), and the total flavonoids (FLOIDS, as mg caffeic acid equivalents Kg-1 oil) antioxidant capacity (as μmol Trolox Kg-1 oil) based on the diphenyl picryl hydrazyl assay (DPPH) and on the ferric reducing power assay (FRAP) were determined according to Roussos et al. (2009).

Soil sampling took place in November from three different places per plot in a "W" pattern (nine soil cores per orchard) by the use of a 5 cm diameter auger to a 30 cm depth after removal of the aboveground biomass. At the same time nearly 40 healthy leaves were collected around the canopy of each sampled tree, at a height of approximately 1-2 m above ground, from non bearing shoots. Leaves were placed into paper bags and transferred to the laboratory, where they were carefully washed with running tap water and three times with de-ionized one, before drying them in an oven at 70 ^{o}C till constant weight and ground then into fine powder. Soil and leaf analysis took place according to Gasparatos and Roussos (2011). Briefly, soil samples were air-dried and ground to 2 mm prior to analysis. Particle size analyses were made using the hydrometer method, with a 2-h reading for clay concentration (Gee and Bauder, 1986). Available metal contents were extracted from the soils by shaking 10 g samples for 2 h with 20 ml 0.005 DTPA (diethylene-triamine-pentaacetic acid) adjusted to pH = 7.3), prepared as described by Lindsay and Norvell (1978). Plant material was dried at 70 ^{o}C for 48 hours, weighted and ground. 0.5g of the plant sample was dry-ashed in an oven at 500 ^{o}C for 4h and the ash was subjected to wet digestion in concentrated nitric acid. Fe, Mn, Cu, Zn concentrations were determined by using a Varian SpectrAA-300 atomic absorption spectrometer. Quality control was assured by duplicate samples and procedural blanks.

Figure 1. Location of trial location.

The trial was conducted as a completely randomized design with four replications and the data were analyzed as a one-way Anova and the significant differences were determined based on Tukey HSD test at a=0.05.

RESULTS AND DISCUSSION

As can be seen in Table 1, the application of attapulgite with or without OMW did not have any significant effect on shoot growth or yield and olive oil production. This could be partly attributed to the short period of time and due to the fact that only a single application was performed per year. This is further supported by the findings of López-Piñeiro et al. (2008)

which observed significant influences of OMW addition after five consecutive years of application. Variable efficacy of OMW application in plants has been reporterd by other researchers, such as Assimakopoulou et al. (2020) working with OMW in pepper plants, where either plant growth remained unaffected or slightly enhanced or even reduced, depending on the dose rate of OMW and the application rate of zeolite added with OMW.

Table 1. The effect of the various treatments on shoot growth and yield components.

Treatments	**Shoot length (cm)**	**Yield (kg/tree)**	**Olive oil per fruit (% w/w)**	**Olive oil per tree (kg)**
Control	15.2 a	65.86 a	24.48 a	16.13 a
Attapulgite	16.4 a	64.16 a	25.58 a	16.25 a
Attapulgite + OMW	16.1 a	59.60 a	26.73 a	15.90 a

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

On the contrary, the application of attapulgite alone resulted in reduced olive oil (OO) acidity compared to other treatments, although the oil produced from all treatments was characterized, based on these indices, as Extra Virgin Olive Oil (EVOO) (Table 2).

Table 2. The effect of the various treatments on olive oil quality indices.

Treatments	**Free acidity (% oleic acid)**	**K232**	**K270**	**ΔK**	**Peroxide value (meq. O_2/kg)**
Control	0.38 a	1.42 a	0.07 a	-0.002 a	10.0 a
Attapulgite	0.26 b	1.68 a	0.07 a	-0.004 b	8.3 a
Attapulgite + OMW	0.33 ab	1.52 a	0.08 a	-0.003 ab	10.0 a

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

There was not any significant influence of any of the treatments tested here, regarding total phenols, total flavonoids and antioxidant capacity of the OO, apart from the detected reduction of total o-diphenols by the presence of attapulgite alone. This was probably the effect of reduced water stress imposed by the application of attapulgite in the soil, as it can reserve water and reduce water losses, relieving partly the olive tree from water stress, which otherwise would increase phenolic compounds to counteract possible reactive oxygen species production. On the other hand, the addition of OMW with attapulgite did not have any significant impact on oil properties, although different results have been reported (Mechri et al. 2009).

The treatments had a significant effect on some of the soil properties as can be seen in Tables 4 and 5. Attapulgite alone increased the concentration of nitrate nitrogen, sodium and boron in the soil (0-30 cm depth), while in combination with OMW it increased phosphorus and manganese concentration. In 30-60 cm depth, attapulgite addition increased the concentration of boron and manganese compared to control, while in combination with OMW it increased the concentration of nitrate nitrogen. Similar results on soil nitrogen, sodium, phosphorus and other nutrients by OMW has been reported by other researchers too (Fernández-Hernández et al. 2014; Magdich et al. 2022), who assigned these changes to increased mineralization though increased microbial activity.

Phosphorus concentration in the leaves increased under the combined effect of attapulgite plus OMW, probably a result of the increased P concentration found in the upper soil level (Table 6). Unlike our present results, Mechri et al. (2011) reported decreases in leaf nutrient levels by the application of OMW, but this difference could be assigned to different soil properties, different cultural management, experimentation conmditions and cultivar.

able 3. The effect of the various treatments on olive oil phenolic compounds and antioxidant capacity.

Treatments	**Total phenols**	**Total o-diphenols**	**Total flavonoids**	**DPPH**	**FRAP**
Control	270.2 a	27.6 a	0.76 a	1100.0 a	1292.9 a
Attapulgite	325.4 a	15.57 b	0.56 a	887.4 a	1000.3 a
Attapulgite + OMW	258.4 a	22.6 ab	0.68 a	1023.6 a	1220.1 a

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

The total phenols are expressed as mg gallic acid equivalents Kg^{-1} oil, the total o-diphenols as mg caffeic acid equivalents Kg^{-1} oil, and the total flavonoids as mg caffeic acid equivalents Kg^{-1} oil, antioxidant capacity (DPPH and FRAP) as μmol Trolox Kg^{-1} oil.

CONCLUSIONS

Based on the present study, it became clear that both the application of attapulgite alone as well as in combination with OMW could be a valuable cultivation practice in improving soil properties and leaf nutrient concentration. Furthermore, the combined application of attapulgite plus OMW could be a method for recycling OMW giving added value to wastes produced during the various agronomic practices. Nonetheless, more research is needed to further examine the long-term effects on olive production and olive oil quality as well as on soil properties and leaf nutrient concentration.

Table 4. The effect of the various treatments on soil physicochemical properties (depth 0-30 cm).

Treatments	pH	$CaCO_3$ (%)	ECe (µS/cm)	OM (%)	N-NO_3	P	Na	K	Ca	Mg	B	Zn	Mn	Cu	Fe
					mg/Kg										
Control	7.54 a	21.6 a	696.8 a	3.18 a	11.46 b	19.9 c	66.6 b	266.6 a	10437 a	503.3 a	0.53 b	0.55 a	8.26 b	1.01 a	6.00 a
Attapulgite	7.54 a	22.8 a	691.2 a	3.42 a	17.43 a	29.2 b	182.0 a	269.3 a	9910 a	443.9 a	0.79 a	0.45 a	6.86 b	0.93 a	5.73 a
Attapulgite + OMW	7.46 a	18.3 a	699.0 a	2.82 a	6.58 b	44.4 a	69.3 b	276.6 a	10495 a	436.8 a	0.65 b	0.52 a	11.7 a	1.03 a	6.01 a

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

Table 5. The effect of the various treatments on soil physicochemical properties (depth 30-60 cm).

Treatments	pH	$CaCO_3$ (%)	ECe (µS/cm)	OM (%)	N-NO_3	P	Na	K	Ca	Mg	B	Zn	Mn	Cu	Fe
					mg/Kg										
Control	7.46 a	20.3 a	722.2 a	2.9 a	6.7 c	33.1 a	78.6 a	166.0 a	10338 a	510.5 a	0.35 b	0.70 a	9.56 b	1.11 a	8.0 a
Attapulgite	7.52 a	21.8 a	720.8 a	2.5 a	11.3 b	34.2 a	92.0 a	171.3 a	10786 a	485.7 a	0.56 a	0.63 a	11.7 a	1.02 a	5.7 b
Attapulgite + OMW	7.45 a	19.8 a	751.0 a	5.9 a	16.7 a	23.2 b	99.3 a	185.3 a	10370 a	497.0 a	0.41 b	0.56 a	9.1 b	1.13 a	7.7 ab

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

Table 6. The effect of the various treatments on leaf nutrient concentration.

Treatments	N	P	K	Ca	Mg	B	Zn	Mn	Cu	Fe
	%					mg/Kg				
Control	1.54 a	0.15 b	0.53 a	1.56 a	0.08 b	13.4 a	25.3 a	30.5 a	21.8 a	71.2 a
Attapulgite	1.62 a	0.18 b	0.66 a	1.45 a	0.08 b	16.0 a	21.7 a	33.2 a	21.1 a	56.7 a
Attapulgite + OMW	1.55 a	0.21 a	0.72 a	2.2 a	0.12 a	13.2 a	23.0 a	32.5 a	24.6 a	61.4 a

Means within the same column followed by the same letter do not statistically differ based on Tukey HSD test at α=0.05.

REFERENCES

Cecchi, L, M., B. Migliorini Zanoni, C. Breschi, N. Mulinacci. 2018. An Effective HPLC-Based Approach for the Evaluation of The Content of Total Phenolic Compounds Transferred From Olives to Virgin Olive Oil During The Olive Milling Process. Journal of the Science of Food and Agriculture. 98 (10):3636-3643.

Chatzistathis, T., T. Koutsos. 2017. Olive Mill Wastewater as A Source of Organic Matter, Water and Nutrients for Restoration of Degraded Soils and for Crops Managed With Sustainable Systems. Agricultural Water Management. 190:55-64.

Commission EU (1991). Regulation EEC 2568/91 On the Characteristics of Olive Oil and Olive Pomace and Their Analytical Methods. Official Journal of European Community. L 248:1-83

Denaxa, N.K., T. Damvakaris, P.A., Roussos. 2020. Antioxidant Defense System in Young Olive Plants Against Drought Stress and Mitigation of Adverse Effects Through External Application of Alleviating Products. Scientia Horticulturae. 259:108812.

Fernández-Hernández, A., A. Roig, N. Serramiá, C.G.O. Civantos, M.A. Sánchez-Monedero. 2014. Application of Compost of Two-Phase Olive Mill Waste on Olive Grove: Effects on Soil, Olive Fruit and Olive Oil Quality. Waste Management. 34(7):1139-1147.

Gasparatos, D., P.A. Roussos, E. Christofilopoulou, C. Haidouti. 2011. Comparative Effects of Organic and Conventional Apple Orchard Management on Soil Chemical Properties and Plant Mineral Content Under Mediterranean Climate Conditions. Journal of Soil Science and Plant Nutrition. 11(4):105-117.

Gee, G.W., J.W. Bauder. 1986. Particle - size analysis. In: A. Klute (ed). Methods of Soil Analysis, Part 1. Physical and Mineralogical Methods. ASA, SSSA, Madison, WI, USA, pp: 383-411.

Lindsay, W.L., W. A. Norvell. 1978. Development of DTPA Soil Test For Zinc, Iron, Manganese and Copper. Soil Science Society America Journal. 42:421-428.

López-Piñeiro, A., A. Albarrán, J.R. Nunes, C. Barreto. 2008. Short and Medium-Term Effects of Two-Phase Olive Mill Waste Application on Olive Grove Production and Soil Properties Under Semiarid Mediterranean Conditions. Bioresource Technology. 99(17):7982-7987.

Magdich, S., B.B. Rouina, E. Ammar. 2022. Combined Management of Olive Mill Wastewater and Compost in Olive Grove: Effects on Soil Chemical Properties at Different Layers Depth. Ecological Engineering.184:106769.

Mechri, B., H. Cheheb, O. Boussadia, F. Attia, F.B. Mariem, M. Braham, M. Hammami. 2011. Effects of Agronomic Application of Olive Mill Wastewater in a Field of Olive Trees on Carbohydrate Profiles, Chlorophyll A Fluorescence and Mineral Nutrient Content. Environmental and Experimental Botany. 71(2):184-191.

Mechri, B., M. Issaoui, A. Echbili, H. Chehab, F.B. Mariem, M. Braham, M. Hammami. 2009. Olive Orchard Amended With Olive Mill Wastewater: Effects on Olive Fruit and Olive Oil Quality. Journal of Hazardous Materials. 172(2-3):1544-1550.

Roussos P, N.K., Denaxa, T. Damvakaris. (2009). Strawberry Fruit Quality Attributes After Application of Plant Growth Stimulating Compounds. Scientia Horticulturae. 119 (2):138-146.

Roussos, P.A., D., Gasparatos. 2009. Apple Tree Growth and Overall Fruit Quality Under Organic and Conventional Orchard Management. Scientia Horticulturae. 123(2):247-252.

THE EFFECT OF BACTERIAL COATING AND STRATIFICATION TEMPERATURE TREATMENTS ON THE GERMINATION OF SEEDS OF ROSE HIP (*Rosa canina* L.)

Mert Kaan YILDIRIM[1], Aysen KOÇ[1]

[1] *Yozgat Bozok University, Agriculture Faculty, Horticulture Department, Yozgat, Türkiye*
Corresponding author e-mail: aysen.koc@yobu.edu.tr

ABSTRACT

This study revealed the effects of different stratification temperatures and bacterial inoculation on the germination success of rosehip seeds belonging to the SRG17 genotype, which is superior in terms of fruit quality among the rosehip genotypes selected from Yozgat province. In the study, single, double and triple combinations of control, EM.A microbial fertilizer, hot and cold stratification treatments were used to increase the germination rates of seeds of the SRG17 genotype. As a result of the study, it was determined that the rosehip seeds germinated 17 days after sowing in the 4th (cold and humid stratification - 150 days), 6th (warm and humid stratification - 4 weeks and cold and humid stratification - 150 days) and 7th (EM.A microbial fertilizer + warm and humid stratification - 4 weeks + cold and humid stratification - 150 days) applications. The best result in terms of germination percentage was obtained with 9.58% from the seeds planted in the 6th application, that is, after 4 weeks of hot and humid stratification and 150 days of cold and humid stratification. In the measurements made on rosehip plants that were germinated and grown until the dormancy period, no statistical difference was found in terms of root collar diameter, stem number and shoot number. In terms of shoot length, the 6th and 7th applications (38.90±3.92 cm and 33.10±16.17 cm, respectively) were found to be statistically different from the 4th application (29.15±10.42 cm). In shoot diameter measurements, the 7th application was found to be statistically different from the other applications with the highest value of 2.49±0.57 mm. In terms of root dry weight, the 6th and 7th applications (7.27±0.65 g and 6.15±2.56 g, respectively) were found to be statistically different from the 4th application (5.46±1.87 g). At the end of the study, it was concluded that the best results on the germination success of different stratification temperatures and bacterial inoculation in the germination of seeds of the SRG17 rosehip genotype were obtained from the combination application of 4 weeks of warm and humid stratification and 150 days of cold and humid stratification.

Keywords: Rosehip, Seed, Stratification temperatures, Bacterial inoculation

INTRODUCTION

Plants have been widely used for centuries to meet a significant portion of people's basic needs such as nutrition, heating and shelter. The rapid increase in the world population, the unconscious use of plant resources to meet the daily needs of people, land clearings, the replacement of native (traditional) varieties with improved varieties, the use of weed killers, the consumption by uprooting from nature instead of production, natural disasters, road and dam constructions, unconscious urbanization and industrialization cause the decrease and rapid loss of plant genetic resources. Both the development of new varieties to increase agricultural production and the transfer of natural (wild) plant species, which are raw materials, to future generations without erosion will be possible through the objective identification, collection and protection of existing plant diversity. (Şehirali and Özgen, 1987; Ercişli and Eşitken, 2004; Tan, 2010).

Rosehip (Rosa spp) is a perennial shrub with many stems and deep roots, upright or climbing form, 0.5 - 4.0 m. tall, although it varies in different species. It has both hairy roots and taproots that go down to 4 m. The fleshy red colored soft roots are used in the dye industry and the eyes on them tend to give root suckers. The leaves are ellipse shaped, toothed, compound leaves with 5 or 7 leaflets. The flowers are pink, yellow, cream, white or light red, with 5 petals and 5 calyxes, have a pleasant smell and a beautiful appearance. However, in some species the number of petals can be much more than 5 like in roses. Rosehip has a hermaphroditic flower structure and has many male and female organs. Its fruit is a false berry. The fruit shape is round, egg shaped or ellipse. The fruit is fleshy, shiny, green before ripening, and varies from brick red to red when ripe. The outer part of the fruit is hairy or hairless depending on the species, the inside of the fruit is more or less hairy and contains many seeds. If not collected, it can remain on the plant in winter. The ripening period of the fruits is between July and October, depending on the species and the climatic conditions of the region (İlisulu, 1992; Türkben, 2003, Güneş, 2013).

Rosehip fruits, which are produced as tea, fruit juice and marmalade, are reported to be especially rich in vitamin C and are the richest plant among cultivated and naturally grown plants (Ağaoğlu et al., 1987). User (1967) reported that since the human body cannot synthesize and store vitamin C, it is necessary to take it daily from outside, that vitamin C ensures the normal formation of collagen, which is an important component of the skin and connective tissues, and bone development, and the healthy development of teeth, that with its antioxidant properties, it neutralizes harmful substances known as free radicals, that it ensures the normal level of cholesterol in the blood, that it helps the body's defense system to function normally by preventing the symptoms that occur in colds and flu, and that it also ensures the use of some vitamins and minerals such as vitamins E, A, B2, B5, folic acid, iron and calcium (Güneş, 2013). Rosehip is used as a raw material in the pharmaceutical industry in many European countries, and its roots and fruits are used in folk medicine in the treatment of hemorrhoids and as a painkiller, and its leaves and flowers are used in the treatment of colds and rheumatism, and bronchitis (Honda et al. 1996).

In fruit species that are pollinated or open to pollination, seed propagation (except for seeds that are formed apomictic) is not a recommended method. Because in seed propagation, it is not possible to obtain a standard fruit variety without losing the variety characteristics. The reason for this is that cross-pollination (allogamy) is common in fruit species and varieties, and as a result, the genetic structures of fruit species show a heterozygous character to a large extent. If the seeds of fruits formed as a result of cross-pollination are used in the production of fruit species, the new individuals formed may not be the same as the mother and father plants in terms of all characters. While this is generally the case in fruit species, the situation is slightly different in the Caninae section of rosehip. Rosehip species show a polyploid structure in terms of chromosome numbers (n=7, 2n=14 can vary from 2n=56 in rosehip species). For example, in rosehips in the Caninae section, the chromosome number can be 2n=28, 35 or 42 depending on the species. In the Caninae section, regardless of the level of chromosome multiples (polyploidy), only 7 chromosomes are transferred from the father plant to the daughter plant during the meiosis division phase. Depending on the polyploidy status, 21, 28 or 35 chromosomes are transferred from the mother plant to the daughter plant. In this case, the daughter plant receives many genetic characters from the mother plant. Therefore, it would not be wrong to say that there is no major opening in seed production in the species of rosehip that fall into the Caninae section. Since the Rosa canina species has a chromosome number of 2n = 35, it will show very little opening when propagated by seed, and this propagation method can be applied (Güneş, 2013).

In our study, the seeds of the SRG17 genotype, which was found to be superior in terms of fruit quality among the rosehip genotypes selected from Yozgat province, were examined and the effects of different stratification temperatures and bacterial inoculation on the germination success of rosehip seeds were revealed.

MATERIAL AND METHOD

The research was conducted at Yozgat Bozok University in 2023. The trial was established in a randomized plot design. In the research, single, double and triple combinations of control, EM.A microbial fertilizer, hot and cold stratification applications were used to increase the germination rates of seeds of the SRG17 genotype (Table 1). The study was carried out with 3 replications and 100 seeds in each replication. A total of 8 applications x 3 replications x 100 seeds = 2400 seeds were used. Seeds were removed from rosehip fruits, cleaned and washed. In all combinations where EM.A would be applied, the seeds were inoculated with bacteria for 20 minutes in microbial fertilizer diluted at a ratio of 1:100 and then taken to stratification. Statistical analysis was performed with SPSS statistical program.

Table 1. Single, double and triple combinations of EM.A microbial fertilizer to be applied to seeds, hot and cold stratification applications

Serial Number	Applications
1	Control
2	Warm and humid stratification (4 weeks)
3	EM.A microbial fertilizer + Warm and humid stratification (4 weeks)
4	Cold and humid stratification (150 days)
5	EM.A microbial fertilizer + Cold and humid stratification (150 days)
6	Warm and humid stratification (4 weeks) + Cold and humid stratification (150 days)
7	EM.A microbial fertilizer + Warm and humid stratification (4 weeks) + Cold and humid stratification (150 days)
8	EM.A microbial fertilizer

In order to break the dormancy, rosehip seeds were stratified in 1 kg plastic containers (20.60 x 11.20 x 6.90 cm) in perlite at a depth of approximately 1 cm. The seeds were then subjected to hot and humid stratification at 25±1 ºC, and then to cold and humid stratification at +4ºC. During the stratification period, the humidity of the stratification media was regularly checked to ensure that the media remained moist. The seeds removed from stratification were planted in peat media.

The first stratification study Hot and humid stratification (4 weeks) + Cold and humid stratification (150 days) application (no. 6) and EM.A microbial fertilizer + Hot and humid stratification (4 weeks) + Cold and humid stratification (150 days) application (no. 7) were carried out on 04.11.2022. The seeds were first placed in water, and those that floated on the water were discarded. The remaining seeds were spread on blotting paper.

In the 6th combination application, the seeds were placed in perlite for hot stratification at room temperature for 4 weeks.

In the 7th combination application, the seeds were first inoculated with bacteria in EM.A diluted at a ratio of 1:100 for 20 minutes and then placed in perlite for hot stratification at room temperature for 4 weeks.

The boxes belonging to both combinations were moistened with water in the form of a spray, with humidity control in between.

On 02.12.2022, the boxes belonging to both combinations were placed in the refrigerator for cold stratification at 4 degrees to be kept for 150 days. These two combinations were kept in cold stratification (150 days) together with all other combinations until they were planted in vials on 05.05.2023.

The second stratification study was carried out on 29.11.2022 in the Cold and moist stratification (150 days) application (no. 4) and EM.A microbial fertilizer + Cold and moist stratification (150 days) application (no. 5). The seeds were first placed in water, and those that floated on the water were discarded. The remaining seeds were spread on blotting paper.

For the 3rd application, the seeds were cold stratified in the refrigerator at 4 degrees to be kept for 150 days.

For the 4th combination application, the seeds were first inoculated with bacteria in EM.A diluted at a ratio of 1:100 for 20 minutes, and the boxes that were stratified were cold stratified in the refrigerator at 4 degrees to be kept for 150 days.

The boxes belonging to both combinations were moistened with water in the form of a spray by checking the humidity in between. These two combinations, along with all other combinations, were kept in cold stratification (150 days) until they were planted in vials on 05.05.2023.

The third stratification study was carried out on 01.04.2023 in the Hot and humid stratification (4 weeks) application (no. 2) and EM.A microbial fertilizer + Hot and humid stratification (4 weeks) application (no. 3). The seeds were first placed in water, and those that floated on the water were discarded. The remaining seeds were spread on blotting paper.

For the application no. 2, the seeds were hot stratified in perlite at room temperature for 4 weeks.

For the combination application no. 3, the seeds were first inoculated with bacteria in EM.A diluted at a ratio of 1:100 for 20 minutes and then hot stratified in perlite at room temperature for 4 weeks.

The boxes of both combinations were moistened with water in the form of a spray, with humidity control in between. These two combinations were kept in hot stratification (4 weeks) with all other combinations until they were planted in the trays on 05.05.2023.

In the fourth and last application, the EM.A microbial fertilizer (no. 8) and Control (no. 1) applications, which were planted without stratification of the seeds, were carried out on 05.05.2023. The seeds were first placed in water, and those that floated on the water were discarded. The remaining seeds were spread on blotting paper.

In the Control application no. 1, the seeds were planted in the vials without any treatment.

For the application no. 8, the seeds were inoculated with bacteria in EM.A diluted at a rate of 1:100 for 20 minutes and then planted in the vials. (Figure 1).

Figure 1. Planting seeds in trays

RESULTS AND DISCUSSION

On 05.05.2023, the seeds planted were regularly checked and the emergence rates of the plants were recorded daily. The first emergences were detected on 22.05.2023. In rosehip plants; germination percentage (%) and germination duration (days) of the applications were determined (Table 2).

Table 2. Germination percentage (%) and germination time (days) of rosehip seeds

Serial Number	Applications	Germination percentage (%)	Germination time (days)
1	Control	0	-
2	Warm and humid stratification (4 weeks)	0	-
3	EM.A microbial fertilizer + Warm and humid stratification (4 weeks)	0	-
4	Cold and humid stratification (150 days)	2,08	17
5	EM.A microbial fertilizer + Cold and humid stratification (150 days)	0,42	17
6	Warm and humid stratification (4 weeks) + Cold and humid stratification (150 days)	9,58	17
7	EM.A microbial fertilizer + Warm and humid stratification (4 weeks) + Cold and humid stratification (150 days)	6,67	17
8	EM.A microbial fertilizer	0	-

The seedlings that reached a size of 5-10 cm (Figure 2) were transplanted into pots prepared with 2 parts of peat + 1 part of perlite on 13.06.2023 and their growth was observed (Figure 3).

Figure 2. Growing rosehip plants from seed

Figure 3. Transplanting germinated and growing rosehip plants into pots

In the dormant period of rosehip plants that were germinated and placed in pots, root collar diameter, stem number, shoot number, shoot length and diameter, root length, root dry and fresh weight, stem dry and fresh weight were determined. The obtained data were subjected to variance analysis using SPSS 25.0 statistical package program, and the differences between the means were checked with Duncan multiple comparison test (Yurtsever, 1984).

In June, regular watering and fertilization were applied to rosehip plants placed in pots in the greenhouse (Figure 4). In plants that shed leaves in December, root collar diameter, stem number, shoot number, shoot length and diameter, root length, root dry and fresh weight, stem dry and fresh weight measurements were made in February.

Figure 4. Rosehip plants placed in pots in the greenhouse

Data on some morphological characteristics detected in the plants from the 4th, 6th and 7th applications that survived the study are given in Table 3.

The root collar diameter in rosehip plants varied between 5.04±1.08 mm (7th application) and 5.17±0.07 mm (6th application). The number of stems was determined as 1.20±0.45 (6th application), 1.40±0.55 (4th application) and 1.60±0.55 (7th application). The number of shoots was counted as 2.40±1.34 (7th application), 2.60±0.55 (6th application) and 2.60±1.14 (4th application). The highest shoot length was measured as 38.90±3.92 cm from the 6th application and 33.10±16.17 cm from the 7th application. The shoot length obtained from the 4th application was statistically in a separate group with 29.15±10.42 cm. In terms of shoot diameter, the 7th application gave the highest value statistically with 2.49±0.57 mm. Shoot diameters of 2.12±0.26 mm and 1.91±0.32 mm were measured in the 4th and 6th applications, respectively.

Table 3. Some morphological characteristics of rosehip plants

Serial No.	Applications	Root collar diameter (mm)	Number of stems (pcs)	Number of shoots (pcs)	Shoot length (cm)	Shoot diameter (mm)
1	Control	0,0 b	0,0 b	0,0 b	0,0 c	0,0 c
2	Warm and humid stratification (4 weeks)	0,0 b	0,0 b	0,0 b	0,0 c	0,0 c
3	EM.A microbial fertilizer + Warm and humid stratification (4 weeks)	0,0 b	0,0 b	0,0 b	0,0 c	0,0 c
4	Cold and humid stratification (150 days)	5,14±1,45 a	1,40±0,55 a	2,60 ±1,14 a	29,15±10,42 b	2,12±0,26 b
5	EM.A microbial fertilizer + Cold and humid stratification (150 days)	0,0 b	0,0 b	0,0 b	0,0 c	0,0 c
6	Warm and humid stratification (4 weeks) + Cold and humid stratification (150 days)	5,17±0,07 a	1,60±0,55 a	2,60±0,55 a	38,90±3,92 a	1,91±0,32 b
7	EM.A microbial fertilizer + Warm and humid stratification (4 weeks) + Cold and humid stratification (150 days)	5,04±1,08 a	1,20±0,45 a	2,40±1,34 a	33,10±16,17 ab	2,49±0,57 a
8	EM.A microbial fertilizer	0,0 b	0,0 b	0,0 b	0,0 c	0,0 c

Root length, root fresh and dry weight, shoot fresh and dry weight data in rosehip plants are given in Table 4.

Root length varied between 13±3.61 cm (4th application) and 17±2.22 cm (6th application). Root fresh and shoot fresh weights were weighed after removing rosehip plants from pots and cleaning roots from peat and perlite residues (Figure 5). Root fresh weight was weighed as 21.72±1.22 g (6th application), 19.29±11.08 g (7th application) and 15.76±5.60 g (4th application). In terms of root dry weight, applications no. 6 with 7.27±0.65 g and no. 7 with 6.15±2.56 g gave the highest statistical value. In application no. 4, root dry weight was weighed as 5.46±1.87 g. Shoot fresh weight was determined as 5.24±1.60 g (7th application), 4.82±0.27 g (6th application) and 4.33±1.16 g (4th application). There was no statistical difference between the germinated plants in terms of shoot dry weight. Shoot dry weight varied between 2.20±0.60 g (4th application) and 2.68±0.84 g (7th application).

Table 4. Root length, root fresh and dry weight, shoot fresh and dry weight data

Serial No	Applications	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)
1	Control	0,0 b	0,0 b	0,0 c	0,0 b	0,0 b
2	Warm and humid stratification (4 weeks)	0,0 b	0,0 b	0,0 c	0,0 b	0,0 b
3	EM.A microbial fertilizer + Warm and humid stratification (4 weeks)	0,0 b	0,0 b	0,0 c	0,0 b	0,0 b
4	Cold and humid stratification (150 days)	13±3,61 a	15,76±5,60 a	5,46±1,87 b	4,33±1,16 a	2,20±0,60 a
5	EM.A microbial fertilizer + Cold and humid stratification (150 days)	0,0 b	0,0 b	0,0 c	0,0 b	0,0 b
6	Warm and humid stratification (4 weeks) + Cold and humid stratification (150 days)	17±2,22 a	21,72±1,22 a	7,27±0,65 a	4,82±0,27 a	2,54±0,17 a
7	EM.A microbial fertilizer + Warm and humid stratification (4 weeks) + Cold and humid stratification (150 days)	15±1,82 a	19,29±11,08 a	6,15±2,56 ab	5,24±1,60 a	2,68±0,84 a
8	EM.A microbial fertilizer	0,0 b	0,0 b	0,0 c	0,0 b	0,0 b

Figure 5. Measurement of fresh and dry weights of roots and stems

CONCLUSIONS

As a result of the study, it was determined that the planted rosehip seeds germinated 17 days after planting in the 4th (Cold and humid stratification - 150 days), 6th (Hot and humid stratification - 4 weeks and Cold and humid stratification - 150 days) and 7th (EM.A microbial fertilizer + Hot and humid stratification - 4 weeks + Cold and humid stratification - 150 days) applications. The best result in terms of germination percentages was obtained with 9.58% from the 6th application, i.e. the seeds planted after 4 weeks of hot and humid stratification and 150 days of cold and humid stratification.

In the measurements made on rosehip plants that were germinated and grown until the dormancy period, no statistical difference was found in terms of root collar diameter, stem number and shoot number. In terms of shoot length, the 6th and 7th applications (38.90±3.92 cm and 33.10±16.17 cm, respectively) were found to be statistically different from the 4th application (29.15±10.42 cm). In shoot diameter measurements, the 7th application was found to be statistically different from the other applications with the highest value of 2.49±0.57 mm. No statistically significant difference was found between the 4th, 6th and 7th applications in terms of root length, root fresh weight, shoot fresh and dry weight. In terms of root dry weight,

the 6th and 7th applications (7.27±0.65 g and 6.15±2.56 g, respectively) were found to be statistically different from the 4th application (5.46±1.87 g).
Studies have shown that rosehip seeds have difficulty germinating due to their hard, impermeable outer shell, and that only one or a combination of pre-treatments may not be sufficient (Foster and Wright, 1983; Tansı et al., 1996; Ercişli, 2000; Belletti et al., 2003, Hoşafcı et al., 2005, Anderson and Byrne, 2007). Among these pre-treatments, stratification has been determined to be an effective method for breaking dormancy when applied hot and cold (Zhou et al., 2009). Another method used to break dormancy in seeds and increase germination percentage is microbial inoculation into seeds or germination medium. It has been reported that microorganisms soften the hard-coated seed pericarp and facilitate germination (Morpeth and Hall, 2000, Kazaz et al., 2010). In our study, no germination occurred in the seeds planted after the application number 1, which was the Control application, which did not have any application, application number 2, which was hot and humid stratification for 4 weeks, application number 3, which was hot and humid stratification for 4 weeks together with EM.A microbial seed coating, application number 5, which was cold and humid stratification for 150 days together with EM.A microbial seed coating, and finally application of only EM.A microbial seed coating. The highest germination was obtained from the combination application of hot and humid stratification for 4 weeks and cold and humid stratification for 150 days. At the end of the study, it was concluded that the best result on the germination success of different stratification temperatures and bacterial inoculation in the germination of seeds of the SRG17 rosehip genotype was obtained from the combination application of hot and humid stratification for 4 weeks and cold and humid stratification for 150 days.

ACKNOWLEDGMENTS

This study was financially supported by TÜBİTAK (Project number: 2209-A, 1919B012221394). We thank to TÜBİTAK for its additions.

REFERENCES

Ağaoğlu, Y. S., Ayfer, M., Fidan Y., Koksal, L, Çelik, M., Abak, K., Çelik, H., Kaynak, L., Gülşen, Y., 1987. Bahçe Bitkileri. A.Ü.Z.F. Yay. 1009, Ofset Basım 31, 281s, Ankara.

Anderson, N., & Byrne, D.H. (2007). Methods for rosa germination. Acta. Hortic. 751, 503-507.

Belletti, P., Cullum, J., Gorian, F., Monteleone, I., & Piotto, B. (2003). The use of a compost activator to overcome seed dormancy in Rosa canina L., In L. Ciccarese, S. Lucci, & A. Mattsson (Eds.), Nursery production and stand establishment of broad-leaves to promote sustainable forest management. (pp. 17-20) Atti 5/2003, APAT.Rome, İtaly.

Ercişli, S. (2000). Farklı uygulamaların kuşburnu tohumlarının çimlenmesi üzerine etkisi. II. Ulusal Fidancılık Sempozyumu, 25-29, Eylül, İzmir Foster ve Wright, 1983.

Ercişli, S. ve Eşitken, A.,2004. Fruit Characteristics of Native Rose Hip (Rosa spp.) Selections From the Erzurum Province of Turkey. New Zealand Journal of Crop and Horticultural Science, vol. 32: 51-53 p.

Güneş, M., 2013. Kuşburnu. Üzümsü Meyveler. Tomurcukbağ Ltd. Şti Eğitim Yayınları No:l, Kalecik /Ankara, sf 423-457.

Honda, G., E. Yeşilada, M. Tabata, E. Sezik, T. Fujita and Y. Takeda, 1996. Traditional Medicine in Turkey VI. Folk Medicine in West Anatolia: Afyon, Kütahya, Denizli, Muğla, Aydın Provinces. J. Ethnopharmacol.53:75-87.

Hosafci, H., Arslan, N., & Sarihan, E.O. (2005). Propagation of dogroses (Rosa canina L.) by seed. Acta Hortic. 690, 159-164.

İlisulu, K., 1992. İlaç ve Baharat Bitkileri. A.Ü.Z.F.Yay. 1250, Ders Kitabı No:360, 302s.
Kazaz, S., Erbaş, S., Baydar, H., 2010. Breaking seed dormancy in oil rose (Rosa damascena Mill.) by microbial inoculation. African Journal of Biotechnology Vol. 9(39), pp. 6503-6508.
Morpeth DR, Hall AM (2000). Microbial enhancement of seed germination in Rosa corymbifera 'Laxa'. Seed Sci. Res. 10: 489-494
Şehirali. S.. Özgen. M.. 1987. Bitki genetik kaynakları. Ankara Üniv. Ziraat Fak. Yayınları No: 1020. Ders Kitabı. 294. Ankara.
Tan. A.. 2010. Türkiye Gıda ve Tarım Bitki Genetik Kaynaklarının Durumu. Gıda ve Tarım için Bitki Kaynaklarının Muhafazası ve Sürdürülebilir Kullanımına İlişkin Türkiye İkinci Ülke Raporu. Ege Tarımsal Araştırma Enstitüsü. Menemen, İzmir. 1-12.
Tansı, L.S. Nacar, Ş, & Çulcu, A.A. (1996). Kuşburnu (Rosa canina) Yetiştirme Olanakları. Kuşburnu sempozyumu Bildiriler Kitabı. 5-6 Eylül Gümüşhane.
Türkben, C., 2003. Kuşburnu. Uludağ Üniversitesi Basımevi, ISBN: 975-6958-70-7, Bursa, 53s.
User, E. T., 1967. Memleketimizde Orta ve Kuzey Anadolu'da yetişen kuşburnunun C vitamini bakımından durumu, bununla ilgili halk gelenekleri hakkında bir araştırma. Türk Hijyen ve Tecrübi Biyoloji Dergisi, Ankara. 27 (1): 39-60.
Zhou, Z.Q., Wei-Kai, B., & Ning, W. (2009). Dormancy and germination in Rosa multibracteata Hemsl. & E.H. Wilson. Sci. Hortic. 119, 434-441.

MORPHOLOGICAL AND DIAGNOSTIC CHARACTERISTICS OF THE PATHOGEN *SPHAEROTHECA PANNOSA VAR.ROSAE* IN ROSE CULTURE

Diellon Salihaj[1]

[1] University "St. Kliment Ohridski" Faculty of Bıotechnıcal Scıence,Bitola

e-mail: diellon.salihaj@uklo.edu.mk

ABSTRACT

Rose ash disease caused by the pathogen Sphaerotheca pannosa var.rosae is one of the most problematic diseases in horticulture, which in all countries of the world causes great financial losses in the field of horticulture. In my country, in Kosovo but also in other Balkan countries, the growth of cultivated areas with this decorative plant is increasing every day.Farmers who cultivate roses have suffered huge losses, precisely because of the presence of this disease in the cultivars that the market is looking for. In our 2-year study, carried out during the 2022-2023 vegetation, we diagnosed the presence of the pathogen Sphaerotheca pannosa var.rosae. The presence of the pathogen was identified by sampling leaves, shoots and flowers, in which a white mold was found.We sent such samples to the plant protection laboratory in the Department of Phytopathology, from where it turned out to be the Pathogen Sphaeroteca pannosa var.rosae.In the laboratory, the vegetative reproductive organs of the pathogen - Conidia with the typical form of this pathogen were identified, where we made their morphological identification, measuring the length and width expressed in micrometers, which we have included in our tabular results. We also identified the intensity of the spread of this disease and found that the pathogen has an increase in virulence from April to September. We have calculated the intensity of the spread of this pathogen according to the Townsend GR and JW Heuberge formula. The 2-year results of this study have shown that protection with adequate fungicides against this disease should continue from the beginning of the vegetation until the end of autumn.

Key words: Disease, Pathogen, Horticulture, Sample, Conidia.

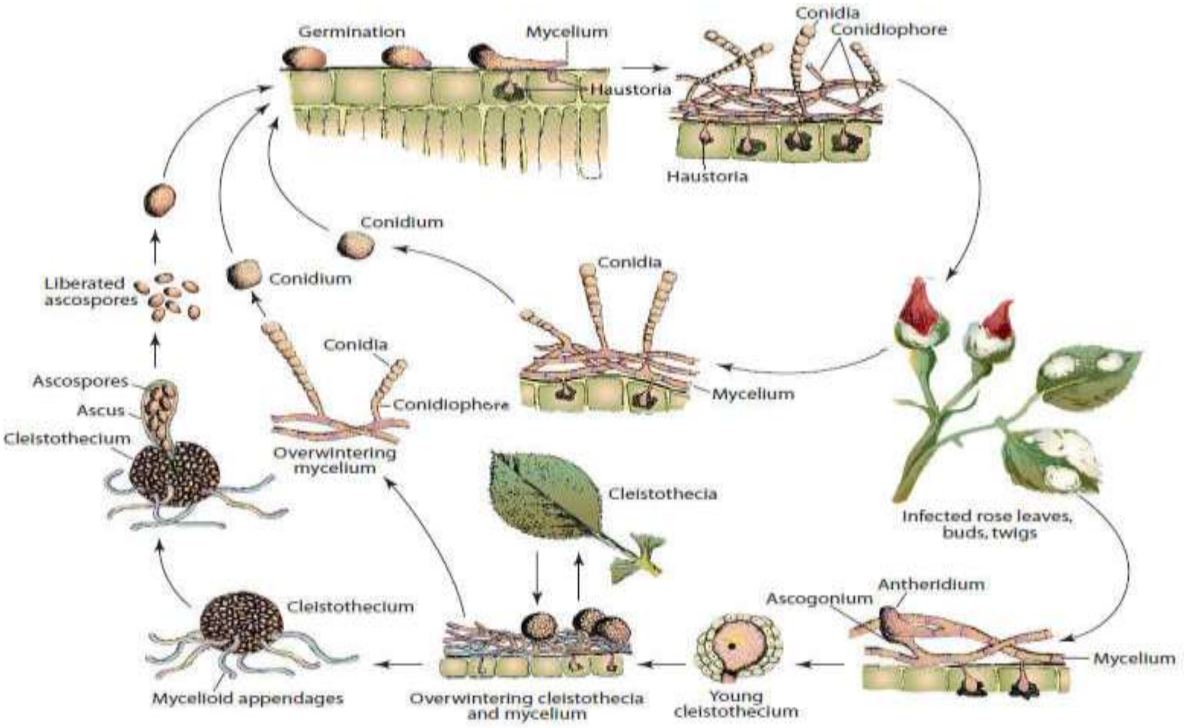
INTRODUCTION

The rose as a decorative plant is cultivated for centuries all over the world, including our country as well, and is increasingly becoming part of the households. The cultivation of this crop besides the necessary agrotechnical conditions, also requires a professional protection from diseases and pests. One of the most common diseases that attack the rose is powdery mildew caused by the pathogen *Sphaerotheca pannosa var.rosae*. The pathogen overwinters in the form of mycelium in infected buds and in the form of generative reproductive organs such as cleistothecium

This disease occurs at the beginning of vegetation, with the emergence of the first buds and continues until the end of vegetation - leaf fall.

When the mycelium overwinters in the rose buds, and the vegetation begins in the spring there is systemic infection on all the leaves that emerge from the infected buds, including the flower The growth of the infected plants is stunted and flowers remain sterile, whereas during the winter the infected stems can not resist low temperatures and as such they perish.

The pathogen overwinters in the dormant buds of the plant as mycelium or as cleistothecium (reproductive organs)
When the pathogen overwinters in the form of cleistothecium the primary infection is caused by ascospores. Throughout vegetation the pathogen forms conidia, which continuously infect the leaves, shoots and flowers of the rose. The climate conditions such as temperature and humidity are of the great importance in infection development.
The optimal temperature for pathogen development is 18-25 ^{o}C. Conidia as vegetative organs of reproduction are formed at a temperature of 21-27 C in a relatively low air humidity (Susuri L Phytopathology 1995).



.

Fig 1.Biological cycle of pathogen *S.Pannosa var,rosae*

MATERIALS AND METHODS

The experiment was conducted during the 2023 vegetation by monitoring this disease from the beginning of the bud opening to the end of vegetation
To indicate the susceptibility of this plant to the pathogen of *Sphaerotheca pannosa var.rosae* the experiment was carried out on chemically untreated cultivars with fungicides
The main objective of this paper was: Diagnostication of the pathogen both macroscopically and microscopically. The measurement of vegetative organs of reproduction – conidia. The occurrence intensity of powdery mildew disease. To accomplish the first objective of this experiment, we sampled the vegetative parts, leaves, rosebuds and flowers of the rose to observe the symptoms of the disease in the infected parts of the plant. Samples were taken twice during vegetation(in the first decade of June and September) from the leaves and flowers in every trunk, ten each.
The second objective of this paper , was the measurement of the vegetative organs of the pathogen.

For obtaining this experiment the taken samples were delivered to the laboratory, to identify microscopically whether the pathogen S.Pannosa is present in the samples.

After morphological identification of the pathogen, we measured the length and width of the conidia in table 1. To accomplish the third objective, we sampled randomly the leaves of the stems.To calculate the index of plant affected by the pathogen we used the *Townsend GR* and *JW Heuberger formula*

$$i = \frac{\sum (n \bullet x)}{5 \bullet N} \bullet 100$$

$\sum$ = Sum of infected leaves by cathegory

n = leaves number of each cathegory

X = certain cathegory

5 = number of cathegory

N = Total number of analyzed leaves

Fig.2.The symptoms of disease caused by pathogen *Sphaerotheca pannosa* ,(Authentic picture)

RESULTS AND DISCUSSION

The observation and measurement of vegetative reproductive organs -conidia of powdery mildew disease caused by *Sphaerotheca pannosa var.rosae* was done from samples of infected leaves and flowers which resulted with clear symptoms of infection(Fig.1). The infected samples were observed in microscope to see the fungal oids that belong to the imperfect form of *Oidium farinosum* pathogen.

The measurement was made with the help of an ocular micrometer, which is a round glass, in the middle of which there is a 5 mm long line, divided into small lines with a distance of 0.1 mm each. The ocular micrometer can be placed in all types of microscopes. To obtain the value of the ocular micrometer, we set the objective micrometer first then proceeded with direct measurements of the reproductive organs of the pathogen - oids. In order to get the results of these measurements we made approximately 50 measurements for each sample, by dividing the number of measurements to get the average of the widths and lengths of the conidia shown in the table below.


Fig.3. Images of oids

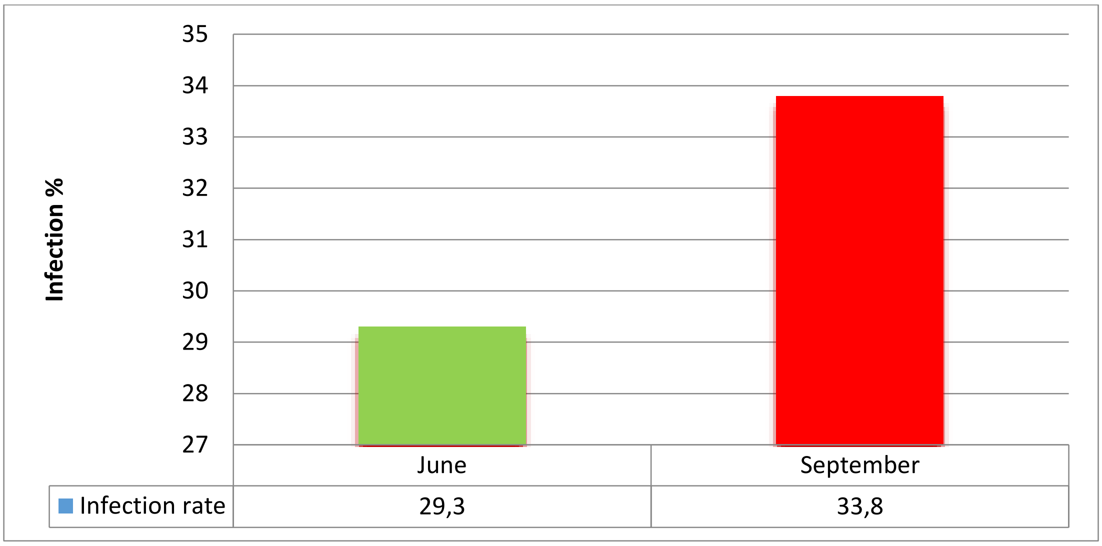
Tab.1 The size measurements of oids *Sphaerotheca pannosa* ***var.rosae***

Sampling time	Observation time	Size (µm-micrometer)
Sampling in June	09. 06. 2023	10,20 - 12.50 x 19,50 - 24,10
Sampling in September	07. 09. 2023	11,30 -13,70 x 21,40 - 26,20

The shown results from the tables indicate that the size of the conids (oids) has changed depending on the study phase.

As can be seen from the table, the size of oids observed at the beginning of June is 10.20 - 12.50 x 19.50-24.10 µm, whereas their size of the same cultivars made in September is 11.30 - 13.70 x 21.40 - 26.20 µm. In the second objective of this paper two assessments were made to determine the intensity of the powdery mildew to rose cultivars caused by *Sphaerotheca pannosa var.rosae*. The first was done in early June and the second in early September by random sampling of 100 leaves from 10 different plants.Then we calculated the index of affected plants by pathogen, by applying the formula of Townsend GR and JW Heuberger. By applying the methodology for achieving this objective the following results were obtained based on years of study.

Tab. 2 Intensity of affectedness of powdery mildew in different sampling stages



CONCLUSIONS

From the study of determining intensity of affectedness with rose powdery mildew caused by Sphaerotheca pannosa var.rosae, we came up with following conclusions: The first symptoms of powdery mildew disease observed by macroscopic and microscopic methods have been noticed by the end of April in the newly emerged leaves from the bud. Later on the disease was noticed in all the green parts of the rose, such as leaves, flowers, and rosebud. During this study it was found that the measured size of oids expressed in micrometer has changed depending on the study stage. The size of oids measured in June were 10.20 - 12.50 x 19.50 - 24.10 µm, while the size made from the same cultivars in September were 11.30 - 13.70 x 21.40 - 26.20 µm. As a result of the assessments it was found that the intensity of the affectedness has increased from June, when the first assessment was done until the second assessment, which was done in September. June assessments were 29.3, compared to 33.8 in September. In this study there were found that first symptoms of S.pannosa appeared from the opening of first buds and subsequently the infection continuously increased to the end of vegetation, which makes this pathogen very dangerous. Also in this study we found that in dry season with no precipitation and high temperatures the disease severity is adversely affected. From the conclusions drawn from this study, we conclude that monitoring of rose powdery mildew disease caused by Sphaerotheca pannosa var.rosae should be done annually to see the susceptibility of the rose to this pathogen. This will serve as a recommendation to farmers who are interested in planting this ornamental crop which is increasingly expanding with new cultivars. Given that the impact of this pathogen is high on untreated plots, it is indispensable for our agro-climatic conditions to carry out more profound studies to see the effectiveness of the pesticides and the most effective point in treating the rose powdery mildew.

REFERENCES

Goifanich G.(1990)ManualeDi Patologia Vegetale Vol ll,pp 515-518 Edizioni Agricole dela Calderini,Via Emilia Levante,31 Bologna

Govi G,(1991) Patologia Vegetale,pp 175-177 Edagricole-Edizioni Agricole delta Calderini,Via Emilia Levante 31 Bologna

Matta A,(1996) Fondamenti di patologia vegetale pp-348,byPatron editore Via Baldini 12 20050 quarto inferior-Bologna
Susuri L, (1995) Fitopatologjia,pp150-153 Universiteti I Prishtines,Kosovë
Salihaj M,(2010) Journal of Internacional Application & Science, volum 5 Nr 1 pp 156-167

N-ACETYLCYSTEINE SUPPRESSES SODIUM FLUORIDE-INDUCED GENOTOXICITY IN MOUSE LEYDIG CELLS

Aleyna AGALAR[1], Yasemin AYDIN[1], Banu ORTA YILMAZ[1,]*

[1] Istanbul University, Science Faculty, Department of Biology, Istanbul, Turkey
**Corresponding author e-mail: banu.yilmaz@istanbul.edu.tr*

ABSTRACT

Fluoride is an essential element for living organisms, found in water, air, and food. Fluoride-containing dental products and fluoridated water are the primary sources of fluoride in the human body. Overexposure to fluoride can harm different organs and tissues, including the skeletal, neurological, digestive, respiratory, and urogenital systems. Reviewing the literature uncovers multiple studies that utilize antioxidant molecules to mitigate the detrimental impacts of fluoride. N-acetylcysteine (Nac), a chemical possessing antioxidant characteristic, directly interacts with oxidative substances to decrease the levels of reactive oxygen species (ROS) upon cellular entry, functioning as a scavenger of reactive oxygen molecules. Nac, which has high antioxidant and anti-inflammatory capacity, is used to treat various diseases related to oxidative damage. With the increasing evidence of fluoride toxicity on the male reproductive system, interest in studies in this direction has increased. However, there is a limited amount of study on the therapeutic properties of Nac in counteracting the harmful impacts of sodium fluoride (NaF) on TM3 Leydig cells. The TM3 Leydig cell line, which is involved in the production of testosterone in the male reproductive system, was exposed to different concentrations of NaF (50 ppm) and Nac (1 mM) for 24 h in this research. The study assessed cell viability and micronucleus formation at specific concentrations of NaF and Nac, focusing on the genotoxic effects of fluoride in the Leydig cell line. Based on the collected results, it was determined that NaF reduced cell viability in Leydig cells and caused genotoxic damage. Furthermore, it was found that the administration of Nac enhanced the survival rate of cells and mitigated the extent of genotoxic damage in Leydig cells that were affected by NaF exposure. Consequently, our study discovered that Nac may possess a therapeutic effect on TM3 Leydig cells in mitigating the harmful effects of NaF toxicity.

Keywords: Fluoride, Leydig cells, n-acetylcysteine, cell viability, genotoxicity.

INTRODUCTION

Fluorine is an environmental pollutant that is widely found in nature and is frequently exposed to by living organisms (Zhao et al., 2022). Fluorine is a highly reactive element due to its high electronegativity. Its low stability causes fluorine to be found in nature as compounds rather than in isolation (Johnston et al., 2020). Fluorine forms compounds mainly with the sodium, aluminum, calcium, and hydrogen, while sodium fluoride can be considered among the most common forms in nature (Han et al., 2021). Fluoridated water and dental products that contain fluoride significantly contribute to the absorption of fluoride in the human body. Fluoride shows its effect on the human body, unlike other pollutants, depending on the concentration. While it has beneficial effects on tooth and bone development below 1 mg/L concentration, according to the World Health Organization report, exposure exceeding the upper limit (1.5 mg/L) causes a health problem known as fluorosis (Pal et al., 2023). Moreover, the utilization of fluoride-contaminated groundwater for irrigating food crops leads to the degradation of plant growth, reduced yield, and subsequent biological accumulation (Koley et al., 2024).

Studies have shown that excessive fluoride exposure damages various organs and tissues in the body, such as the skeletal, nervous, digestive, respiratory, and urogenital systems (Lacson et al., 2021). Numerous toxicology studies reveal adverse effects of fluoride toxicity, including reduced male reproductive capacity, decreased sperm quality, changes in testicular organ weight, and histopathological changes in reproductive tissues (Cao et al., 2016; Li et al., 2018). In addition, excessive fluoride consumption negatively affects sperm characteristics, fertility, and reproductive hormone levels. In another study, it was observed that NaF caused some abnormalities in the reproductive system and also induced oxidative stress by producing reactive oxygen species (ROS) in the sperm and liver of mice. Accordingly, it was observed that fluoride affected DNA, lipids, proteins, and enzymatic systems (Yu et al., 2018). When genotoxicity studies were examined, it was seen that high fluoride intake disrupted the cell cycle in various tissues and cells, induced apoptosis, and caused DNA damage (Herrera-Calderon et al., 2019).

The World Health Organization considers N-acetylcysteine (Nac) as an essential drug and uses it as a mucolytic agent in respiratory diseases (Seyedasgari et al., 2024). Nac, a plant antioxidant found in some food products, is the precursor of glutathione (Liu et al., 2021). In addition, NAC protects the activity of biological macromolecules by reacting with reactive oxygen species (ROS) via sulfhydryl groups while contributing to the strengthening of intracellular defense through the synthesis of glutathione (GSH) (Pan et al., 2022; Seyedasgari et al., 2024). Examining studies on the male reproductive system reveals that NAC positively impacts spermatogenesis and testicular function. Accordingly, it is also thought that NAC can be used in the treatment of diseases caused by oxidative stress in the male reproductive system (Tenório et al., 2021). Another study reported that infertile men treated with NAC had improved sperm count, sperm motility parameters, and sperm chromatin integrity (Seyedasgari et al., 2024).

This study selected Leydig cells as a model to investigate the harmful effects of NaF, a prevalent fluorine component in nature, on the male reproductive system. The genotoxicity induced by the administration of NaF on Leydig cells, which are crucial for testosterone production and have a significant impact on the male reproductive system, was assessed using the micronucleus test. Furthermore, the potential of Nac to mitigate NaF-induced genotoxicity was examined for the first time in Leydig cells.

MATERIAL AND METHODS

Cell Culture

The TM3 Leydig cells utilized in the investigation are non-tumorigenic cells derived from the testicular tissues of mice aged 11–13 days. The cells were purchased from the American Type Culture Collection (Manassas, VA, USA). The Leydig cells were cultured in a medium consisting of a 50:50 mixture of DMEM-F12, supplemented with 5% horse serum and 2.5% fetal bovine serum. The cells were cultured in a sterile incubator at a temperature of 37 °C with a controlled atmosphere of 5% CO_2 and 95% air. Two different doses of NaF (10 and 50 ppm) were administered to TM3 Leydig cells for 24 h. Among the two concentrations, the 50 ppm NaF concentration exhibited a significant reduction in cell viability, specifically by 63.56%. Therefore, the 50 ppm NaF concentration was chosen for further experimentation. Furthermore, the Nac concentration selected for this investigation was decided to be 1 mM, based on *in vitro* studies that have demonstrated its antioxidant effects at this concentration. For the final situation, the experiment consisted of four distinct groups: control, NaF, Nac, and NaF+Nac.

Cell Viability

The viability of Leydig cells was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) kit from Roche, Germany. The effects of NaF (10 and 50 ppm) and Nac (1 mM) on Leydig cell viability were investigated. The cells were placed at a density of $5x10^3$ cells per well in 100 µL of assay media in a 96-well plate. Following the incubation period, 10 µl of MTT I solution was introduced into each well, and the plates were subsequently incubated for 4 h at a temperature of 37 °C in a CO_2 incubator. Subsequently, a volume of 100 µl of MTT II solution (SDS) was added to each well and left to incubate overnight in a CO_2 incubator. The ELISA equipment was used to measure absorbances at a wavelength of 540 nm. The untreated control cells were considered to have 100 % viability. The viability of the groups treated with NaF and Nac was expressed as a percentage relative to the control. The experiments were repeated three times.

Cytokinesis-blocked micronucleus (CBMN) assay

The experiment was conducted following the method described by Fenech (2007), with some modifications. TM3 Leydig cells were grown in 6-well plates at a density of $1.5x10^5$ cells/mL and allowed to adhere overnight. The cells were exposed to NaF at a concentration of 50 ppm and Nac at a concentration of 1 mM for 24 h. After the exposure period, the experimental medium was taken out, and the cells were treated with a medium containing cytochalasin B at a final concentration of 4 µg/mL for 20 h. After being harvested, the cells were promptly centrifuged, the liquid portion was separated, and the cells were subsequently mixed with a hypotonic solution containing 1% formaldehyde and 0.075 M KCl. The mixture was then left to incubate for 5 min. After being treated with Cornay's reagent (3:1 methanol/glacial acetic acid), the cell suspensions were centrifuged again. Subsequently, the cell solution was dropped onto slides and dried using air. The slides were immersed in a solution of 4% Giemsa in phosphate buffer (composed of Na_2HPO_4 (0.06 M) and KH_2PO_4 (0.06 M), with a pH of 6.8) for 8 min. For the purpose of determining the occurrence of micronucleus (MN), cytoplasmic bridges (NPBs), and/or nuclear buds (NBUDs), a brightfield microscope (Olympus IX71) was employed to examine each slide and record the results. Two independent scorers assessed the microscope slides in a blinded and random manner. The number of mononucleated and binucleated cells containing MN, NPBs, and/or NBUDs was determined at a minimum of 1000 cells per concentration.

Statistical analysis

Descriptive statistics for the values were performed using GraphPad Prism 9 software (GraphPad Software, San Diego, CA, USA). The Shapiro-Wilk test was employed to assess the normality of the values, and it was determined that the results followed a normal distribution. Therefore, the experiment utilized one-way analysis of variance with Tukey's multiple comparison test for all analyses. The threshold for statistical significance was established at $p<0.05$, $p<0.01$, and $p<0.001$ for all tests.

RESULTS

Cytotoxicity results

Figure 1 displayed the cell viability rates (%). After 24 hours, there was a noticeable drop in MTT values between the control group and the NaF group due to the increasing levels of NaF ($p<0.01$, $p<0.001$). When comparing the group exposed to NaF alone with the group treated with both NaF and Nac, a substantial rise in MTT values was observed in the NaF+Nac groups ($p<0.01$, $p<0.001$). From the cell viability data we acquired, it was evident that NaF had a cytotoxic effect on Leydig cells. However, it was established that Nac might serve as a potent antioxidant to ameliorate the detrimental impact of NaF on Leydig cells.

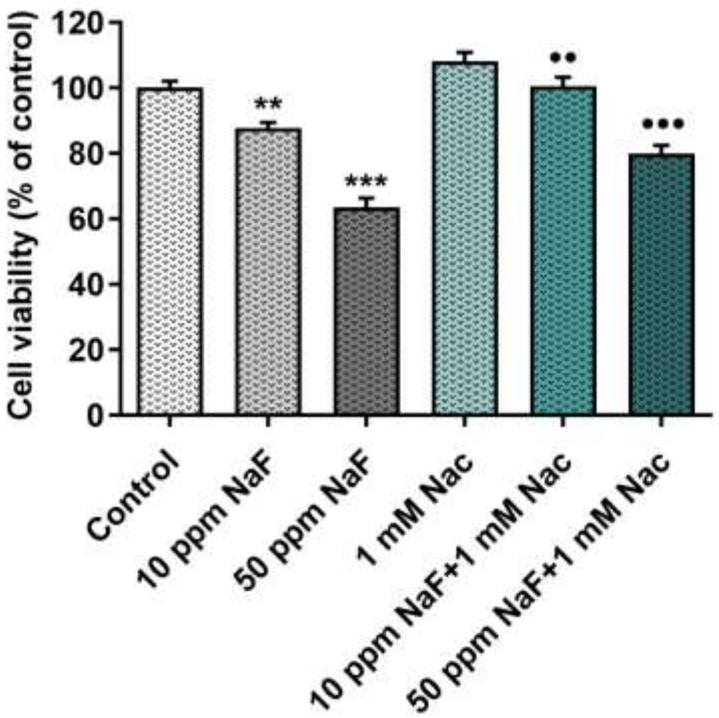


Figure 1. Effects of NaF and Nac on the cell viability in Leydig cells. (**$p<0.01$; ***$p<0.001$; *: compared with control; •: compared with NaF groups).

Genotoxicity results

Figure 2 represents the appearance of micronuclei, bud formation, and cytoplasmic bridges generated by NaF and Nac in Leydig cells, and Table 1 contains the results of analyzing these parameters by comparing them to the control group. The administration of a 50 ppm NaF concentration resulted in a statistically significant increase in binucleated and mononucleated cells with micronuclei and bud compared to the control group ($p<0.001$). The NaF+Nac group showed significantly lower micronucleus rates and bud formation than the NaF alone group ($p<0.001$). Examination of binucleated cells revealed a significant rise in cytoplasmic bridge formation in the groups treated with 50 ppm NaF ($p<0.05$), whereas no protective effect of Nac was noticed.

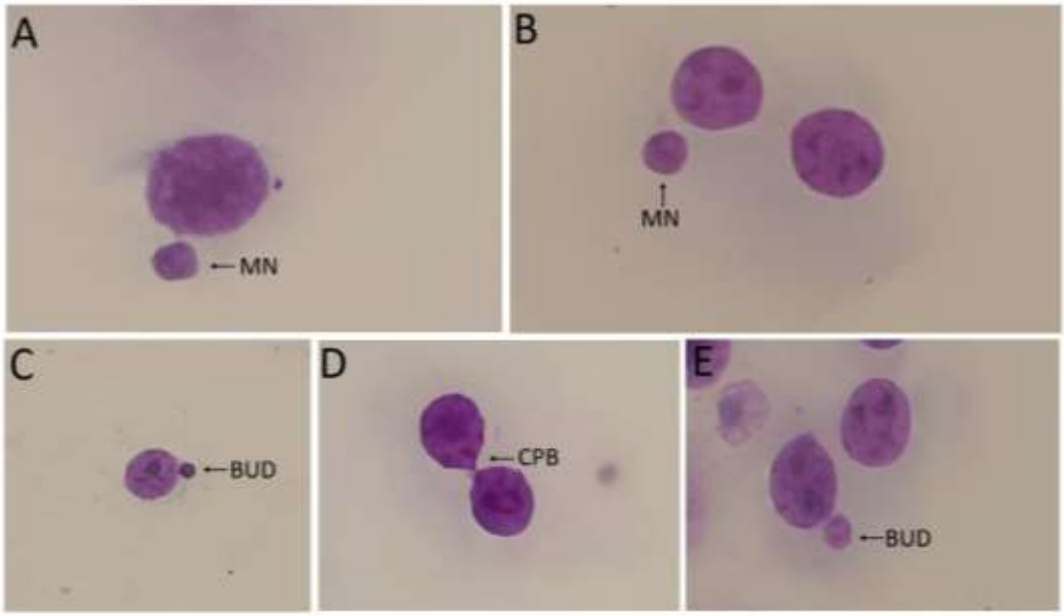


Figure 2. Nuclear abnormalities observed in Leydig cells exposed to NaF and Nac as a result of the micronucleus test. A: Micronucleus in a mononucleated cell; B: Micronucleus in a binucleated cell; C: Bud in a mononucleated cell; D: Cytoplasmic bridge in a binucleated cell; E: Bud in a binucleated cell.

Table 1. Micronucleus test findings for NaF and Nac treated TM3 Leydig cells

	Mononucleated cells		**Binucleated cells**		
	MN	Bud	MN	Bud	CPB
Control	0.47±0.1	1.22±0.1	0.25±0.1	0.90±0.1	1.55±0.1
NaF	6.03±1.1***	3.43±0.8**	5.86±1.1***	3.82±0.3***	2.12±1.2*
Nac	0.51±0.1	0.76±0.1	0.06±0.0	0.33±0.0	0.90±0.1
NaF+Nac	1.36±0.2•••	1.71±0.6•••	1.26±0.3•••	1.09±0.2••	2.16±0.8
PC (H_2O_2)	31.74±1.7	7.38±1.1	29.29±1.2	12.40±1.0	45.47±2.1

Each result corresponds to the mean value obtained from three separate experiments that were conducted three times. MN: micronuclei; CBP: cytoplasmic bridge, PC: positive control. *In comparison to the control, • in comparison to NaF (*$p<0.05$, **$p<0.01$, ***$p<0.001$).

DISCUSSION

Studies have proven that excessive NaF exposure has many harmful effects on organisms (Johnston et al., 2020; Zhao et al., 2022; Ghosh and Ghosh, 2019). Studies on the male reproductive system have shown that NaF leads to disorders by altering the structure of the epididymis and testis and reducing serum testosterone levels (Pal et al., 2023; Yu et al., 2018; Cao et al., 2016). Determining acceptable levels of NaF requires a thorough understanding of these detrimental consequences. The critical role of Nac, which has strong antioxidant properties, in reducing the harmful effects of NaF on Leydig cells was examined for the first time in this study.

Previous investigations revealed that NaF diminished cell viability in various cell lines (Yu et al., 2018; Zhang et al., 2024; Jiang et al., 2020; Yang et al., 2015). In a study, to determine the effect of NaF on cell viability, cell viability was measured in Leydig cells in the presence of increasing NaF concentrations (0, 5, 10 and 20 mg/L) for 24, 48, 72, 96 and 120 hours. The study conducted by Song et al. (2014) showed that exposure to NaF at concentrations of 10 and 20 mg/L had a strong inhibitory effect on the proliferation of Leydig cells. To validate the detrimental effects of fluoride on Leydig cells, the cells were exposed to higher concentrations of NaF (1.2 and 4 mM), following a similar approach as the previous work. Based on the MTT results, the cell viability showed a significant decrease in the groups treated with 1, 2, and 4 mM NaF (Li et al., 2024). In another study, cytotoxicity caused by fluoride varnishes used for the prevention of caries in dentistry practices was measured in human gingival fibroblast cells (hGF). The metabolic activity of hGFs after exposure to different concentrations of fluoride varnishes was analyzed by MTT. According to the results of the study, it was determined that the metabolic activity of hGFs decreased significantly after exposure to fluoride varnishes compared to the control groups (López-García et al., 2021). In one of the studies conducted to demonstrate the protective effect of Nac, it was revealed that Nac played an important role in suppressing cytotoxicity against patulin (PAT)-induced cytotoxicity using human embryonic kidney cells (HEK293). Compared to the PAT applied alone groups, Nac was found to increase cell viability by 102.07%, 389.48% and 412.21% at concentrations of 2, 4 and 10 mM, respectively (Liu et al., 2021). A study with spermatogenic cells focused on the ameliorative role of Nac against malathion toxicity. Malathion caused a significant decrease in the viability of spermatogenic cells at different concentrations (1 and 100 nM) compared to the control group. Nac was found to have a significant ameliorative role against both malathion concentrations (Bhardwaj et al., 2018). In a study with Sertoli cells, it was observed that cells exposed to NaF decreased cell viability, while Nac protected against cell viability (Yang et al., 2015). Consistent with other research, this in vitro investigation utilizing Leydig cells revealed that a concentration of 50 ppm NaF resulted in decreased cell viability. However, the presence of Nac offered substantial defense against the harmful effects of NaF.

Genotoxicity means the loss of DNA integrity as a result of adverse effects occurring in the genetic material of the cell (Savale, 2018). Today, many methods are used to determine genotoxic damage. The micronucleus test is commonly preferred in scientific studies. A laboratory experiment using human bone cells demonstrated that when the NaF concentration was 100 µg/ml, a statistically significant increase was observed in the number of binucleated cells containing micronuclei and nucleoplasmic bridges at 48 hours of exposure compared to the control. Volobaev et al. (2020) observed a considerable increase in the number of binucleated cells having micronuclei, nucleoplasmic bridges, and nuclear protrusions when the NaF content in the culture medium was 200 µg/ml, compared to the control group. In a study using zebrafish erythrocytes, the frequency of micronucleated erythrocytes (%), as determined by the micronucleus test, was shown to be considerably higher on days 30 and 60 of exposure to F. The administration of fluoride also resulted in the occurrence of additional nuclear abnormalities, including the formation of segmented, lobed, and notched nuclei in erythrocytes (Mondal et al., 2023). In another study, the micronucleus assay was used to evaluate the effect of Nac on ionizing radiation (IR)-mediated genotoxicity. It was observed that 10 Gy IR increased the number of micronuclei, which was significantly reduced by Nac (Kurashige et al., 2016). Consistent with the previously described research, our findings revealed that 50 ppm NaF increased micronucleus, bud, and cytoplasmic bridge formation, while 1 mM Nac reduced these nuclear abnormalities and potentially ameliorated genotoxic damage.

In conclusion, this study evaluated the therapeutic effect of Nac against toxic effects caused by NaF using Leydig cells as an in vitro model. In our study, it was determined that NaF significantly suppressed cell viability and induced genotoxicity in Leydig cells. It was found that Nac could protect Leydig cells from genotoxicity induced by NaF. Thus, Nac effectively prevented MN production and subsequently protected against cellular damage induced by NaF.

FUNDING

This study was supported by Istanbul University Scientific Research Projects (Project No. 40289).

REFERENCES

Bhardwaj, J. K., Saraf, P., Kumari, P., Mittal, M., & Kumar, V. (2018). N-Acetyl-cysteine mediated inhibition of spermatogonial cells apoptosis against malathion exposure in testicular tissue. *Journal of Biochemical and Molecular Toxicology*, *32*(4), e22046.

Cao, J., Chen, Y., Chen, J., Yan, H., Li, M., & Wang, J. (2016). Fluoride exposure changed the structure and the expressions of Y chromosome related genes in testes of mice. *Chemosphere*, *161*, 292-299.

Fenech, M. (2007). Cytokinesis-block micronucleus cytome assay. *Nature protocols*, *2*(5), 1084-1104.

Ghosh, S., & Ghosh, D. (2019). Impact of fluoride toxicity on freshwater fishes: A mini-review. *International Journal of Advance Research and Innovation*, *6*(2), 13-18.

Han, J., Kiss, L., Mei, H., Remete, A. M., Ponikvar-Svet, M., Sedgwick, D. M., ... & Soloshonok, V. A. (2021). Chemical aspects of human and environmental overload with fluorine. *Chemical Reviews*, *121*(8), 4678-4742.

Herrera-Calderon, O., Chacaltana-Ramos, L., Yuli-Posadas, R. Á., Pari-Olarte, B., Enciso-Roca, E., Tinco Jayo, J. A., ... & Franco-Quino, C. (2019). Antioxidant and cytoprotective effect of piper aduncum L. against sodium fluoride (NaF)-induced toxicity in albino mice. Toxics, 7(2), 28.

Jiang, N., Guo, F., Sun, B., Zhang, X., & Xu, H. (2020). Different effects of fluoride exposure on the three major bone cell types. *Biological Trace Element Research*, *193*, 226-233.

Johnston, N. R., & Strobel, S. A. (2020). Principles of fluoride toxicity and the cellular response: a review. *Archives of toxicology*, *94*(4), 1051-1069.

Koley, R., & Mondal, N. K. (2024). Synthesis of calcium-based nanofertilizer and its efficacy towards reduction of oxidative stress and fluoride uptake in rice (Oryza sativa L.). *Plant Nano Biology*, 100087.

Kurashige, T., Shimamura, M., & Nagayama, Y. (2016). Differences in quantification of DNA double-strand breaks assessed by 53BP1/γH2AX focus formation assays and the comet assay in mammalian cells treated with irradiation and N-acetyl-L-cysteine. *Journal of radiation research*, *57*(3), 312-317.

Lacson, C. F. Z., Lu, M. C., & Huang, Y. H. (2021). Fluoride-containing water: A global perspective and a pursuit to sustainable water defluoridation management-An overview. Journal of Cleaner Production, 280, 124236.

Li, J., Shi, Y., Fan, H., Li, Y., Zhu, Y., Lin, X., & Zhang, J. (2018). Effects of fluoride on surface structure of primary culture Leydig cells in mouse. Biological trace element research, 183, 123-127.

Li, X., Yang, J., Shi, E., Lu, Y., Song, X., Luo, H., ... & Zhang, J. (2024). Riboflavin alleviates fluoride-induced ferroptosis by IL-17A-independent system Xc−/GPX4 pathway and iron metabolism in testicular Leydig cells. *Environmental Pollution*, *344*, 123332.

Liu, J., Liu, Q., Han, J., Feng, J., Guo, T., Li, Z., ... & Peng, X. (2021). N-Acetylcysteine inhibits patulin-induced apoptosis by affecting ROS-mediated oxidative damage pathway. *Toxins*, *13*(9), 595.

López-García, S., Pecci-Lloret, M. P., Pecci-Lloret, M. R., Guerrero-Gironés, J., Rodríguez-Lozano, F. J., & García-Bernal, D. (2021). Topical fluoride varnishes promote several biological responses on human gingival cells. *Annals of Anatomy-Anatomischer Anzeiger*, *237*, 151723.

Mondal, P., Mukhopadhyay, D., Shaw, P., Dey Bhowmik, A., & Chattopadhyay, A. (2023). Environmentally relevant fluoride alters nuclear integrity in erythrocytes and induces DNA damage in hepatocytes of zebrafish. *The Nucleus*, *66*(1), 1-9.

Pal, P., Jha, N. K., Pal, D., Jha, S. K., Anand, U., Gopalakrishnan, A. V., ... & Mukhopadhyay, P. K. (2023). Molecular basis of fluoride toxicities: beyond benefits and implications in human disorders. *Genes & Diseases*, *10*(4), 1470-1493.

Pan, N., Gao, K., Zhang, B., Fan, X., Lu, L., & Wang, X. (2022). Inhibitory effects of zinc chloride (ZnCl2), n-acetyl-L-cysteine (NAC), and calcium/calmodulin dependent protein kinase II inhibitor (KN93) on Cd2+-induced abnormal cell morphology and membrane permeability. *Science of The Total Environment*, *833*, 155208.

Savale, S. K. (2018). Genotoxicity of drugs: Introduction, prediction and evaluation. *Asian Journal of Biomaterial Research*, *4*(6), 1-29.

Seyedasgari, F., Asadi, B., & Kim, E. (2024). The effect of different concentrations of N-acetyl cysteine on liquefaction, quality and fertility of chilled semen in dromedary camel. *Small Ruminant Research*, *230*, 107166.

Song, G. H., Wang, R. L., Chen, Z. Y., Zhang, B., Wang, H. L., Liu, M. L., ... & Yan, X. Y. (2014). Toxic effects of sodium fluoride on cell proliferation and apoptosis of Leydig cells from young mice. *Journal of physiology and biochemistry*, *70*, 761-768.

Tenório, M. C. D. S., Graciliano, N. G., Moura, F. A., Oliveira, A. C. M. D., & Goulart, M. O. F. (2021). N-acetylcysteine (NAC): impacts on human health. *Antioxidants*, *10*(6), 967.

Volobaev, V. P., Serdyukova, E. S., Kalyuzhnaya, E. E., Schetnikova, E. A., Korotkova, A. D., Naik, A. A., ... & Larionov, A. V. (2020). Investigation of the genotoxic effects of fluoride on a bone tissue model. *Toxicological Research*, *36*, 337-342.

Yang, Y., Huang, H., Ba, Y., Cheng, X. M., & Cui, L. X. (2015). Effect of oxidative stress on fluoride-induced apoptosis in primary cultured Sertoli cells of rats. *International journal of environmental health research*, *25*(1), 1-9.

Yang, Y., Lin, X., Huang, H., Feng, D., Ba, Y., Cheng, X., & Cui, L. (2015). Sodium fluoride induces apoptosis through reactive oxygen species-mediated endoplasmic reticulum stress pathway in Sertoli cells. *Journal of Environmental Sciences*, *30*, 81-89.

Yu, Y., Han, Y., Niu, R., Wang, J., Manthari, R. K., Ommati, M. M., & Sun, Z. (2018). Ameliorative effect of VE, IGF-I, and hCG on the fluoride-induced testosterone release suppression in mice Leydig cells. *Biological trace element research*, *181*, 95-103.

Zhang, Q., Li, T., Shi, R., Qi, R., Hao, X., & Ma, B. (2024). Fluoride promotes the secretion of inflammatory factors in microglia through NLRP3/Caspase-1/GSDMD pathway. *Environmental Science and Pollution Research*, *31*(13), 19844-19855.

Zhao, H., Zhu, Y., Zhao, Y., Wang, T., Li, H., Yang, J., ... & Wang, J. (2022). Alleviating effects of selenium on fluoride-induced testosterone synthesis disorder and reproduction toxicity in rats. *Ecotoxicology and Environmental Safety*, *247*, 114249.

MULTI-TRAIT SELECTION INDEX FOR SIMULTANEOUS SELECTION OF WINTER BARLEY GENOTYPES

Boryana Dyulgerova
Institute of Agriculture – Karnobat, Agricultural Academy, Bulgaria

e-mail: bdyulgerova@abv.bg

ABSTRACT

This study aims to assess winter barley genotypes for the selection of desirable ideotypes based on their grain yield and yield-related characteristics. Field trials were conducted using a Complete Block Design with four replications at the Institute of Agriculture – Karnobat, Bulgaria, during the 2021/2022 and 2022/2023 growing seasons. The traits measured included spike number per m^2, plant height, lodging, spike length, spikelet number per spike, grain number per spike, grain weight per spike, 1000-grain weight, and grain yield. A significant effect of genotype, year, and their interaction was observed ($p < 0.01$) for the performance of 21 barley advanced breeding lines and varieties across the nine traits. Moderate to high broad-sense heritability ($30\% < H^2 \leq 60\%$) was noted for all traits except for lodging and grain yield ($H^2 < 30\%$). The ranking of genotypes by the multi-trait genotype–ideotype distance index (MGIDI) varied between the two years. The advanced line G12 was identified as a promising genotype in both years, indicating its potential for inclusion in multi-environment trials for testing as a candidate variety. The promising genotypes selected based on their performance for multiple traits closest to the ideotype could be further utilized in winter barley breeding programs targeting grain yield improvement.

Kaywords: *Hordeum vulgare* H., Multi-Trait Genotype–Ideotype Distance Index, Yield, Yield-Related Traits

INTRODUCTION

The escalating global demand for grain, driven by population growth and dietary shifts, has intensified pressure on barley production. As a fundamental component of malting, brewing, and livestock feed, barley plays a pivotal role in food security and economic stability. Developing barley varieties that combine high yield with desirable agronomic and quality traits is a complex challenge for breeders. Traditional selection methods, often focused on individual traits, have limitations in capturing the intricate relationships between multiple characteristics. A cornerstone of crop improvement, multi-trait selection aims to enhance multiple desirable traits simultaneously. While this approach holds immense potential, it is fraught with challenges. Genetic correlations between traits, the complexities of assigning economic weights, and the influence of environmental factors on genotype performance significantly complicate the selection process. Moreover, phenotypic evaluation, data analysis, and the achievement of optimal genetic gain across multiple traits demand advanced methodologies.

To address this complexity, researchers have explored various selection indices (Sanhueza et al., 2002; Vieira et al., 2016; Rocha et al., 2018). A promising advancement in this area is the multi-trait genotype-ideotype distance index (MGIDI), introduced by Olivoto and Nardino (2021). By considering multiple traits simultaneously and employing factor analysis to account for trait interdependencies, MGIDI offers a robust approach to genotype selection. The MGIDI is determined using BLUPs, which can remove environmental variance and deliver precise estimates of individual breeding values. Consequently, BLUPs are increasingly favored by

plant breeders for accurate genotypic value estimation (Triki et al., 2023). Moreover, this method is not affected by the issue of multi-collinearity (Olivoto and Nardino, 2021). Its successful application in crops such as wheat (Pour-Aboughadareh and Poczai, 2021; Dastfall et al., 2024), rice (Jalalifar et al., 2023; Palaniyappan et al., 2024), maize (Singamsetti et al., 2023), and oat (Klein et al., 2023) underscores its potential as a valuable tool for accelerating crop improvement.
This study aims to assess winter barley genotypes for the selection of desirable ideotypes based on their grain yield and yield-related characteristics.

MATERIALS AND METHODS

Twenty-one six-rowed winter barley (*Hordeum vulgare* L.) genotypes were included in the study, comprising the Bulgarian national standard variety Veslets (G1), along with the varieties Izgrev (G2), IZ Bori (G3), Bozhin (G4), Zemela (G5), and sixteen advanced breeding lines (G6 to G21)
The study was conducted over two growing years, 2021/2022 and 2022/2023, at the experimental field of the Institute of Agriculture-Karnobat, Southeastern Bulgaria (42°39' N, 26°59' E). The soil of the experimental field was slightly acidic (pH 6.2) Pellic Vertisol. The experiments were designed using a complete block with four replications on 10 m^2 plots, with a sowing rate of 450 germinated seeds per m^2. Standard technology for growing winter barley breeding materials at the Institute was employed.
The plant height (PH, cm), spike length (SL, cm), number of spikelets per spike (NSS), number of grains per spike (NGS), and grain weight per spike (GSW, g) were measured on 20 randomly selected plants in each replication of each genotype. The number of spikes per m^2 (SPM) was determined by courting of spikes before harvest in 0.25 m^2 area from the middle of plots and converted to 1 m^2. Lodging was rated on a scale 9-1, where 9 indicates a high level of resistance to lodging. Grain yield (GY, t ha^{-1}) and 1000-grain weight (TGW, g) were determined on a plot basis.
Data analysis and graph construction were performed using R (version 4.3.0) in the R Studio integrated development environment. Two-way analysis of variance (ANOVA) was used to identify significant differences among the genotypes and growing seasons. The genetic parameters and multi-trait genotype-ideotype distance index (MGIDI) were calculated using the R package 'metan' (Olivoto and Lúcio, 2020). Heritability estimates are classified as low (5-10%), medium (10-30%) and high (30-60%) (Dabholkar, 1992).

RESULTS AND DISCUSSION

Analysis of Variance and Genetic Parameters

The result of combined analysis of variance (Table 1) revealed highly significant variation ($p < 0.001$) between genotype, year, and genotype by year interaction for all studied traits.
The lowest value of phenotypic variance (Vph) was observed for grain weight per spike, whereas the highest was recorded for the number of spikes per m^2 (Table 2). Broad-sense heritability (H^2) estimates were high for spike length and 1000-grain weight, moderate for the number of spikes per m^2, plant height, spikelet number, grain number per spike, and grain weight per spike, and low for lodging and grain yield. Heritability on the mean basis (h^2_{mg}) was moderate to high for most of studied traits. Traits with high heritability should be prioritized for direct genetic selection, while those with moderate and low heritability may benefit from a

combination of genetic and environmental management strategies. The accuracy (Ac) of selection was low for lodging, moderate for grain yield, and high for the rest of the studied traits. Genotype-environment correlation (rge) exhibited high values for most of the traits, indicating the significant role of both genetic and environmental factors in trait expression and performance. The highest value for the coefficient of relative variation (CV ratio) for 1000-grain weight implies that genetic differences are the main source of variation for this trait. As a result, selecting for higher 1000-grain weight will likely produce substantial changes in this trait, making direct selection an effective strategy for improving 1000-grain weight in breeding program.

Table 1. Mean squares from combined analysis of variance for yield-related traits of 21 barley genotypes assessed for two growing seasons

Traits	GEN	ENV	GEN x ENV	Residuals
NSM	19843.10*	4505.36*	6836.60*	36.17
PH	111.18*	12428.72*	43.06*	2.24
L	2.90*	49.29*	2.27*	0.28
SL	6.85*	45.26*	1.07*	0.04
SNS	230.76*	9884.11*	71.57*	1.11
GNS	221.93*	9446.83*	62.00*	0.57
GWS	0.37*	3.74*	0.08*	0.03
TGW	61.06*	778.26*	15.36*	0.04
GY	5.34*	55.22*	3.21*	0.45

* significant level at $p \leq 0.01$; NSM – number of spikes per m^2; PH - plant height, cm; L – lodging (score 9-1), SL - spike length, cm; SNS - spikelet number of per spike; GNS - grain number of per spike, GWS - grain weight per spike, g; TGW - 1000- grain weight, g; GY - grain yield, t ha^{-1}

Table 2**.** Genetic parameters for grain yield and yield-related traits in winter barley genotypes

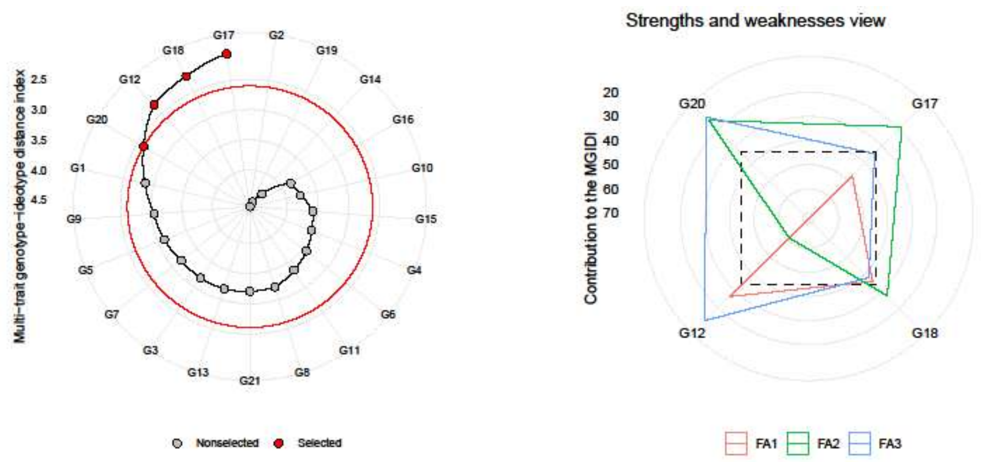
Parameters	NSM	PH	L	SL	SNS	GNS	GWS	TGW	GY
Vph	3361.42	20.80	0.83	1.02	38.63	35.90	0.07	9.58	1.34
H^2	0.48	0.41	0.09	0.71	0.52	0.56	0.50	0.60	0.20
GEIr2	0.51	0.49	0.61	0.25	0.46	0.43	0.18	0.40	0.53
h^2mg	0.66	0.61	0.22	0.84	0.69	0.72	0.79	0.75	0.40
Ac	0.81	0.78	0.47	0.92	0.83	0.85	0.89	0.87	0.63
rge	0.98	0.84	0.67	0.88	0.94	0.97	0.37	0.99	0.66
CVg	9.37	2.73	3.57	12.48	7.18	8.74	8.62	5.53	8.13
CVr	1.38	1.33	6.34	2.74	1.70	1.44	6.88	0.48	9.49
CV ratio	6.79	2.05	0.56	4.56	4.23	6.07	1.25	11.58	0.86

V_{ph} - phenotypic variance; H^2 - heritability in broad sense; GEIr2 - coefficient of determination of the interaction effects; h^2_{gm} - heritability on the mean basis; Ac – accuracy of selection; r_{ge} - genotype-environment correlation, CVg- genotypic coefficient of variation; CVr - residual coefficient of variation; CV ratio - ratio between genotypic and residual coefficient of variation; NSM – number of spikes per m^2; PH - plant height, cm; L – lodging (score 9-1), SL - spike length, cm; SNS - spikelet number of per spike; GNS - grain number of per spike, GWS - grain weight per spike, g; TGW - 1000- grain weight, g; GY - grain yield, t ha^{-1}

Multi-Trait Genotype–Ideotype Distance Index (MGIDI)

In the 2021/2022 growing season, four breeding lines—G17, G18, G12, and G20—were ranked higher than the standard variety Veslets (G1), applying a 20% selection intensity (Figure 1). The right part of Figure 1 displays the strengths and weaknesses of the selected genotypes. The figure indicates that genotype G12 had strengths related to FA2, suggesting that this breeding line exhibited high values for spikelets and grain number per spike, grain weight per spike, and lodging resistance. The minimal contribution of FA2 for genotype G17 indicates that this genotype had the highest number of spikes per square meter and the highest grain yield for that growing year. Both G20 and G12 exhibited strengths related to FA3, suggesting these genotypes possessed a favorable combination of plant height, spike length, and 1000-grain weight.

Figure 1. Ranking of genotypes based on the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) for 2021/2022 growing season



In the 2022/2023 growing season, variety Zemela (G5) and the breeding lines G7, G10, and G12 were selected (Figure 2). The strengths and weaknesses view of the selected genotypes shows that G5 and G10 had strengths connected to FA1, indicating high spike productivity as a result of high spikelet and grain number and grain weight per spike. The lowest contribution of FA2 for genotype G7 suggests that the genotype is characterized by a high number of spikes per square meter, shorter stems, and long spikes. G12 exhibited strengths related to FA3, suggesting this genotype possessed a favorable combination for grain yield and lodging resistance in this growing year.

The ranking of genotypes based on MGIDI fluctuated significantly between the two study years, underscoring a pronounced genotype-by-environment interaction. Notably, G12 consistently emerged as a top-performing advanced line among the selected genotypes, highlighting its adaptability across varying environmental conditions.

The selection differential and selection gain observed in the MGIDI were negative for studied yield-related traits (Table 2). These findings may be attributed to the prioritization of grain yield, suggesting a potential negative relationship between the studied yield-related traits and actual grain yield.

The MGIDI index achieved the desired selection differential (SD) for all studied traits except plant height in 2021/2022 (Table 3) and for all traits except plant height and the number of spikes per m² in 2022/2023 (Table 4). Despite the undesired positive gains in plant height in both growing years, the index still showed substantial genetic improvements. The most significant genetic gain was observed for grain weight per spike, with selection differentials (SD) of 11.10% in 2021/2022 and 13.60% in 2022/2023.
The less significant gains for other traits can be attributed to the simultaneous selection of multiple traits, which tends to reduce the genetic gain for each trait individually (Almeida et al., 2021). However, the reduction in individual trait gains can be offset by the overall genetic gains across the set of traits. In present study, the MGIDI index delivered total gains of 43.37% in 2021/2022 and 62.24% in 2022/2023 for direct selection, considering traits with desired positive gains. This indicates that the MGIDI index is an effective tool for selecting genotypes with the desired characteristics.

Figure 2. Ranking of genotypes based on the Multi-Trait Genotype-Ideotype Distance Index (MGIDI) for 2022/2023 growing season

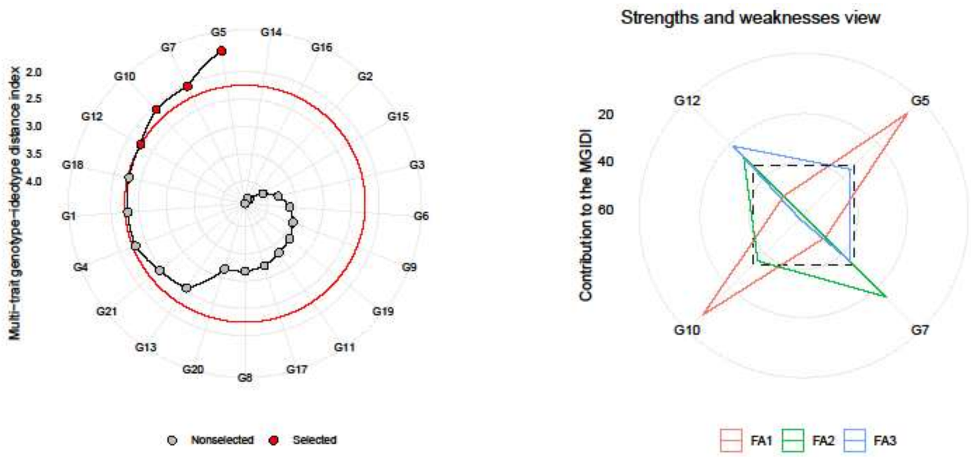


Table 3. Selection differential of the MGIDI index for grain yield and yield-related traits in barley genotypes for 2021/2022 growing season

VAR	Factor	Xo	Xs	SD	SDperc	SG	SGperc
L	FA1	7.89	8.13	0.24	2.59	0.18	2.28
SNS	FA1	54.45	57.63	3.18	5.69	3.02	5.55
GNS	FA1	43.66	45.72	2.06	4.65	2.00	4.59
GWS	FA1	2.06	2.31	0.25	11.10	0.21	10.00
NSM	FA2	435.60	450.13	14.53	3.33	14.50	3.32
GY	FA2	5.78	6.01	0.23	3.94	0.22	3.78
PH	FA3	98.48	103.00	4.52	4.52	4.38	4.45
SL	FA3	6.29	6.68	0.39	6.01	0.37	5.88
TGW	FA3	45.39	48.13	2.74	6.04	2.74	6.03

Table 4. Selection differential of the MGIDI index for grain yield and yield-related traits in barley genotypes for 2022/2023 growing season

VAR	Factor	Xo	Xs	SD	SDperc	SG	SGperc
SNS	FA1	69.79	79.17	9.38	13.40	9.37	13.40
GNS	FA1	58.65	67.22	8.57	14.60	8.56	14.60
GWS	FA1	2.35	2.72	0.36	13.60	0.29	12.10
NSM	FA2	425.85	410.06	-15.78	-3.69	-15.70	-3.68
PH	FA2	115.68	116.91	1.23	1.02	1.13	0.98
SL	FA2	7.33	7.85	0.52	6.99	0.51	6.97
TGW	FA2	41.08	41.59	0.51	1.25	0.51	1.25
L	FA3	7.81	7.94	0.13	1.50	0.11	1.38
GY	FA3	6.92	7.68	0.76	10.90	0.67	9.70

CONCLUSIONS

The combined analysis of variance indicated highly significant variations among genotypes, years, and their interaction for all the studied traits. Most traits showed moderate to high heritability (30% < $H^2 \leq$ 60%), except for lodging and grain yield (H^2 < 30%). Genotype rankings based on MGIDI were influenced by year-to-year environmental variation. Advanced line G12 demonstrated consistent high performance across both years, establishing it as a promising candidate for further cultivar development. The identified superior genotypes, exhibiting traits aligned with the ideal plant type, hold potential for enhancing grain yield in future winter barley breeding programs.

ACKNOWLEDGEMENTS

The author thank the Bulgarian Ministry of Education and Science for the support of the National Research Programme “Smart crop production” approved by Decision of the Ministry Council №866/26.11.2020 г.

REFERENCES

Almeida, G. Q., J. R. A. S. Rocha, H. P. Pessoa, F. M. Alves, L. J. Chaves. 2021. Selection of Hancornia speciosa germplasm accessions based on the FAI-BLUP index. Pesquisa Agropecuaria Tropical 51: e67226.

Dabholkar, A. R. 1992. Elements of Biometrical Genetics, Concept Publishing Company, New Delhi, p. 431.

Dastfall, M., T. Najafi Mirak, H. Zali. 2024. Selection of durum wheat (Triticum turgidum L. var. durum) genotypes tolerant to terminal season drought stress using multi-trait indices (MGIDI). Iranian Journal of Crop Sciences, 25(4): 342-361.

Jalalifar, R., A. Sabouri, S. Mousanejad, A. R. Dadras. 2023. Estimation of Genetic Parameters and Identification of Leaf Blast-Resistant Rice RILs Using Cluster Analysis and MGIDI. Agronomy, 13(11): 2730.

Klein, L. A., V. S. Marchioro, M. Toebe, T. Olivoto, D. Meira, C. Meier, G. Benin, A. C. Busatto, D. C. Garafini, J. V. Alberti, J. L. B. Finatto. 2023. Selection of superior black oat lines using the MGIDI index. Crop Breeding and Applied Biotechnology, 23: e45112332.
Olivoto, T., A. D. C. Lúcio. 2020. Metan: An R package for multi environment trial analysis. Methods in Ecology and Evolution, 11: 783–789.
Olivoto, T., M. Nardino. 2021. MGIDI: Toward an effective multivariate selection in biological experiments. Bioinformatics, 37(10): 1383-1389.
Palaniyappan, S., P. Arunachalam, S. Banumathy, S. Muthuramu. 2024. Introspection of discriminate function analysis and MGIDI selection index for selection to improve yield in rice (Oryza sativa L.). Indian journal of genetics and plant breeding, 84(02): 202-208.
Pour-Aboughadareh, A., P. Poczai. 2021. Dataset on the use of MGIDI index in screening drought-tolerant wild wheat accessions at the early growth stage. Data in Brief, 36: 107096.
Rocha, J. R. D. A. S. D. C., J. C. Machado, P. C. S. Carneiro. 2018. Multitrait index based on factor analysis and ideotype-design: Proposal and application on elephant grass breeding for bioenergy. GCB Bioenergy, 10(1):52-60.
Sanhueza, R. P., T. L. White, D. A. Huber, A. R. Griffin. 2002. Genetic parameter estimates, selection indices and predicted genetic gains from selection of Eucalyptus globulus in Chile. For. Genet., (9):19–29.
Singamsetti, A., P. H. Zaidi, K. Seetharam, M. T. Vinayan, T. Olivoto, A. Mahato, K. Madankar, M. Kumar, K. Shikha. 2023. Genetic gains in tropical maize hybrids across moisture regimes with multi-trait-based index selection. Frontiers in Plant Science, 14: 1147424.
Triki, T., L. Bennani, F. Boussora, S. Tlahig, S. Ben Ali, A. Gasmi, H. Yahia, K. Belhouchette, M. Loumerem, F. Guasmi. 2023. Characterization and Trait Association Analysis of 27 Pearl Millet Landraces in Southern Tunisia. Agronomy, 13:2128.
Vieira, R. A., R. Rocha, C. A. Scapim, A. T. Amaral, M. Vivas. 2016. Selection index based on the relative importance of traits and possibilities in breeding popcorn. Genet. Mol. Res., 15:1–10.

PROTECTIVE EFFECTS OF N-ACETYLSYSTEINE ON SODIUM FLUORIDE-INDUCED CYTOTOXICITY AND APOPTOSIS IN MOUSE LEYDIG CELLS

Yağmur YILMAZ[1], *Öykü KÜMBÜL*[1], *Banu ORTA YILMAZ*[1], *Yasemin AYDIN*[1,*]

[1] *Istanbul University, Science Faculty, Department of Biology, Istanbul, Turkey*

Corresponding author e-mail: yastun@istanbul.edu.tr

ABSTRACT

Fluoride is a naturally occurring element that is abundantly found in nature and belongs to the halogen group. Fluoride compounds are encountered by individuals in several ways on a daily basis, including through the consumption of drinking water, food, and dental products. Research has shown that fluoride has detrimental effects on human health and can lead to toxicity. Furthermore, clinical research and animal trials have conclusively established that fluoride exerts harmful effects on the male reproductive system. Upon reviewing the literature, researchers have conducted numerous studies using antioxidant molecules to enhance the detoxifying effects of fluoride. N-acetylcysteine (Nac) is an amino acid that is produced from L-cysteine and has antioxidant effects. Nac has been globally utilized for more than five decades and is included in the World Health Organization's list of essential medicines. Scientific evidence has demonstrated that Nac effectively safeguards cells and tissues from oxidative stress by eliminating reactive oxygen radicals (ROS). Additionally, researchers have discovered that Nac shields cells from apoptosis by influencing genes involved in programmed cell death. The investigation involved individual and combined application of sodium fluoride (NaF) (50 ppm) and Nac (1 mM) to the TM3 Leydig cell line for a duration of 24 hours. We assessed the cytotoxicity in Leydig cells using the lactate dehydrogenase (LDH) test after exposure and determined apoptosis using the double fluorescence staining method. The findings demonstrated that sodium fluoride induced cytotoxicity in Leydig cells and resulted in apoptosis. Furthermore, studies have established that Nac, renowned for its antioxidant properties, could potentially shield cells from the damaging effects of sodium fluoride.

Keywords: Fluoride, Leydig cells, n-acetylcysteine, apoptosis, cytotoxicity.

INTRODUCTION

Fluorine, a nonmetal in the halogen group, is an environmental contaminant. Fluorine, as an element, does not naturally exist in ionized form, and readily combines with other elements to generate fluoride compounds (Mikkonen et al., 2018; Su et al., 2020). The primary fluoride compounds consist of sodium fluoride (NaF), aluminum fluoride (AlF_3), and calcium fluoride (CaF_2) (Ashoori et al., 2022). Fluoride, an exogenous chemical, is commonly present in drinking water (Chaudhari et al., 2024). Furthermore, the chemical, plastic, and petroleum sectors utilize fluoride compounds that are naturally present in plants and foods (Veneri et al., 2023). Exceeding the limit of 1.5 ppm of fluoride intake, as stated by the World Health Organization, leads to toxicity in both humans and animals (Banerjee and Roychoudhury 2019).

Fluoride has been associated with a range of harmful effects, including the development of diseases such as cancer, brain damage, Alzheimer's, thyroid disorders, and infertility. Research indicates that fluoride triggers oxidative stress, inflammatory responses, and programmed cell death mechanisms such necrosis and apoptosis (Long et al 2003; Rahmani et al., 2020; Veneri et al., 2023; Guo et al., 2024; Taher et al., 2024). Fluoride exposure has been found to result in a decline in male fertility, as observed by Liang et al. in 2020. Fluoride induces testicular oxidative damage in the male reproductive system, leading to damage to the sperm cell membrane. It has been noted that it contributes to the suppression of sperm production and reduced sperm function (Öncü et al., 2007). Several studies indicate that exposure to fluoride leads to a decline in sperm quality, as well as a drop in sperm count and motility (Long et al., 2009; Wang et al., 2009; Chaithra et al., 2018). Research conducted on Leydig cells has shown that exposure to fluoride leads to the production of ROS, an elevation in lactate dehydrogenase levels, damage to mitochondria, and the initiation of apoptosis (Liang et al., 2020). Fluoride exposure is recognized to induce an elevation in reactive oxygen species levels by inhibiting the production of intracellular agents that scavenge free radicals (Zhang et al., 2023). A study investigating the impact of fluoride on testicular toxicity found that prolonged exposure to fluoride led to an increase in the formation of superoxide anion, which in turn had a detrimental effect on the mitochondrial membrane potential. Fluoride exposure has been found to reduce the activities of enzyme antioxidants, such as catalase, superoxide dismutase, and glutathione peroxidase, in the testis. This exposure also affects sperm quality and morphology, leading to toxicity (Radovanovic et al., 2022).

N-acetylcysteine (Nac) is a compound that is derived from L-cysteine, which is a naturally occurring amino acid. Nac, which occurs naturally in meat, fish, grains, dairy products, soybeans, and eggs, possesses anti-inflammatory, antimicrobial, antiapoptotic, and antioxidant properties (Schwalfenberg, 2021; Zhou et al., 2021). Initially, Nac was employed as a therapeutic intervention for individuals suffering with cystic fibrosis, owing to its ability to dissolve mucus. Furthermore, Nac has been frequently utilized as a therapeutic agent to counteract paracetamol overdose (Herzenberg, 2019). Nac has been found to possess several notable qualities, such as the ability to restore glutathione levels, enhance the activity of glutathione S-transferase, stabilize protein structures, and eliminate free radicals (Schwalfenberg, 2021). A study found that the injection of Nac prevented NaF-induced apoptosis. Based on this investigation, Nac has demonstrated a therapeutic impact on apoptosis (Zhang et al., 2023). Studies investigating the effects of Nac on the male reproductive system have shown that it elevates sperm concentration and volume. Additionally, it has been found to decrease sperm DNA oxidation (Zhou, 2021).

This study utilized Leydig cells as a model to demonstrate the harmful effects of sodium fluoride on the male reproductive system. The application of NaF and Nac to Leydig cells, which play a crucial role in testosterone production in the male reproductive system, resulted in the use of the lactate dehydrogenase (LDH) test to measure cell toxicity, and the double fluorescence staining method to detect apoptosis. Thus, we have investigated for the first time whether Nac plays a therapeutic role on NaF-induced cell damage, using TM3 Leydig cells.

MATERIAL AND METHODS

Cell Culture Condition and Treatments

The TM3 cell line employed in the experiment is a non-tumorigenic line derived from Leydig cells of 11-13-day-old mice. We brought it to our laboratory from the American Type Culture Collection (ATCC): The Global Bioresource Center, where we grow it under *in vitro* conditions through regular passages two to three times a week. The cells are grown in 50:50 DMEM/F12 culture medium supplemented with 5% horse serum, 2.5% fetal bovine serum, 2.5

mM L-glutamine, 0.5 mM sodium pyruvate, 1.2 g/L sodium bicarbonate, 15 mM HEPES, and PSA (penicillin, streptomycin, and amphoterin) and incubated at 37ºC in a humidified environment containing 5% CO_2 and 95% air.

This study involved the setting up of several groups, including a control group, a group treated with sodium fluoride (NaF), a group treated with N-acetyl cysteine (Nac), and a group treated with both fluoride and N-acetyl cysteine. NaF concentration was determined as 50 ppm, while Nac concentration was determined as 1 mM. Following the administration of fluoride and Nac to TM3 Leydig cells, the cytotoxicity was assessed using the LDH test, while apoptosis was determined using the double fluorescence staining method.

Determination of cytotoxicity by measuring Lactate Dehydrogenase enzyme (LDH)

LDH is an intracellular enzyme located in the cytoplasm of cells. LDH is released into the external environment when the cell membrane is damaged. The death of cells in a culture or harm to the plasma membrane results in an elevation in LDH enzyme activity. Initially, LDH catalyzes the conversion of lactate into pyruvate. Through this process, NAD+ undergoes reduction and is transformed into NADH/H+. During the second stage, the catalyst substance facilitates the conversion of a tetrazolium salt from NADH/H+ to the red formazan product of H/H+. The quantity of pigment produced is directly proportional to the extent of harm inflicted against the cellular membrane. Each well of the 96-well culture plates was inoculated with 10,000 cells. NaF and Nac concentrations were made in a medium containing 1% HS serum. The samples were then applied for 24 hours and incubated in a 37°C CO2 incubator. After the experiment period concluded, a 100 µl mixture of dye and enzyme solutions was added to each well of the kit. The results were obtained by measuring the absorbance of the increasing color of the red formazan product after 30 minutes of incubation. This was done using a spectrophotometer at a wavelength of 492 nm to assess the cytotoxicity. Once the absorbance readings were acquired, the percentage of the resulting toxicity was computed.

Determination of apoptosis with Double Fluorescent Staining (Propidium Iodide/Hoechst 33342) Method

The approach of double fluorescence labeling was employed to ascertain the occurrence of apoptosis in cells. The Ho342 fluorescent dye specifically attaches to adenine-thymine-rich regions that are exposed in fragmented DNA, allowing it to label apoptotic cells in a blue color. The experimental mixture was generated by combining 20 µl of Ho342 with 3960 µl of PBS, obtained from solutions of Hoechst 33342 (1 mg/ml) and PBS (1M, pH 7.4), respectively. We seeded 10,000 cells per well in 24-well culture dishes. We treated the samples with NaF and Nac solutions that were produced in a medium containing 1% HS serum. The samples were incubated in a 37°C CO2 incubator for 24 hours. After the experimental period, the cells were rinsed with a PBS solution. Then, 0.2 ml of the experimental combination was introduced into each well and left to incubate for 15–30 minutes at a temperature of 37 °C. After the incubation period, the cells were rinsed with PBS one to two times and seen using a fluorescence microscope. The specimens were analyzed using an Olympus IX71 fluorescence microscope equipped with a suitable filter. They were then photographed sequentially at regular intervals using an Olympus DP72 video camera.

Statistical Analysis

In the evaluation of statistical analysis, firstly data distribution was tested for normality using Shapiro-Wilk test. Then, one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test was used for comparisons between all groups. Results were expressed as mean ± standard error and $p<0.05$ was considered statistically significant. All statistical analyses were performed using GraphPad Prism 10 software (GraphPad Software, San Diego, California, USA).

RESULTS

Cytotoxicity results

The effect of NaF on cell toxicity was investigated using the LDH kit 24 hours after fluoride was applied to TM3 Leydig cells separately and together with Nac. The cytotoxicity rates (%) obtained are shown in Figure 1. When the control and NaF groups were compared in terms of LDH values, a significant increase was observed in the group exposed only to NaF compared to the control group at the end of 24 hours. When the LDH values of the group exposed only to NaF and the group applied together with Nac were compared, a significant decrease was observed in the NaF+Nac group. Based on the cytotoxicity data we obtained, it was understood that NaF has a cytotoxic effect on Leydig cells. In addition, it was determined that Nac could be used as a very effective antioxidant molecule to improve this negative effect of NaF on Leydig cells.

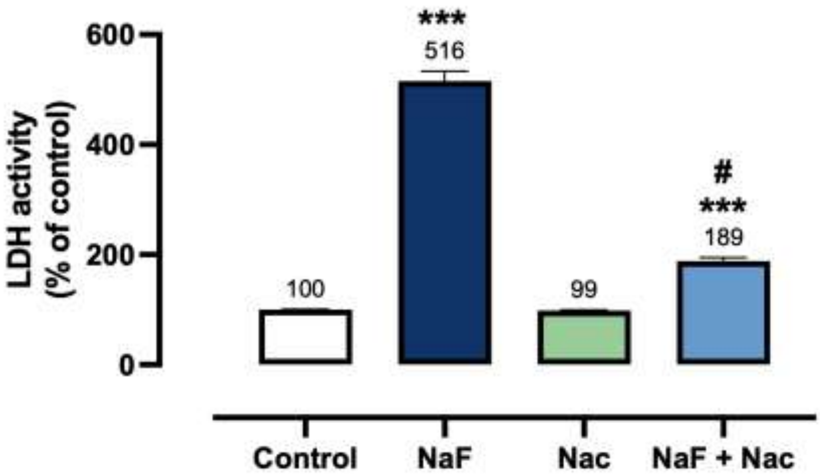


Figure 1. Effects of NaF and Nac on cytotoxicity in TM3 Leydig cells. (*p<0.05; **p<0.01; ***p<0.001; *: compared with controls; #: compared with fluoride group).

Apoptosis results

The percentage of live and apoptotic cells determined using the fluorescent staining method in the experimental groups formed with NaF and Nac in TM3 Leydig cells are presented in Figures 2 and 3, and diagnostic photographs are presented in Figure 4. After the experimental period, the apoptosis rates were compared between the control group and the NaF group. The NaF group showed a substantial rise in apoptosis rates, whereas there was a significant decrease in live cell rates ($p<0.001$). When comparing the group that just administered NaF with the group that applied Nac in addition to NaF, a notable drop in the rate of apoptosis was detected in the NaF + Nac group, while a significant rise in the rate of live cells was observed ($p<0.001$).

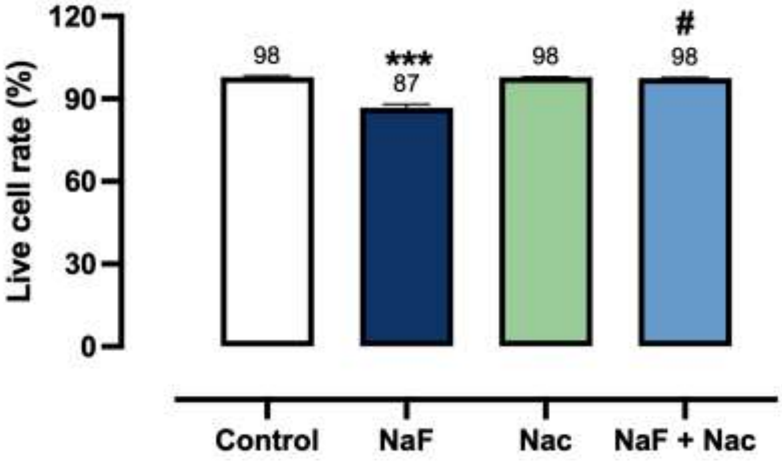


Figure 2. Effects of NaF and Nac on live cell rate in TM3 Leydig cells. (*p<0.05; **p<0.01; ***p<0.001; *: compared with controls; #: compared with fluoride group).

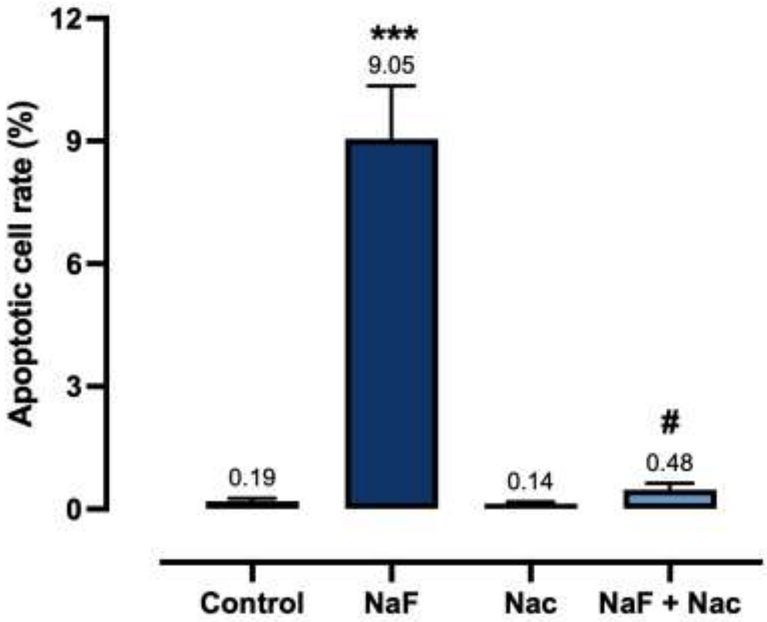


Figure 3. Effects of NaF and Nac on apoptotic cell rate in TM3 Leydig cells. (*p<0.05; **p<0.01; ***p<0.001; *: compared with controls; #: compared with fluoride group).

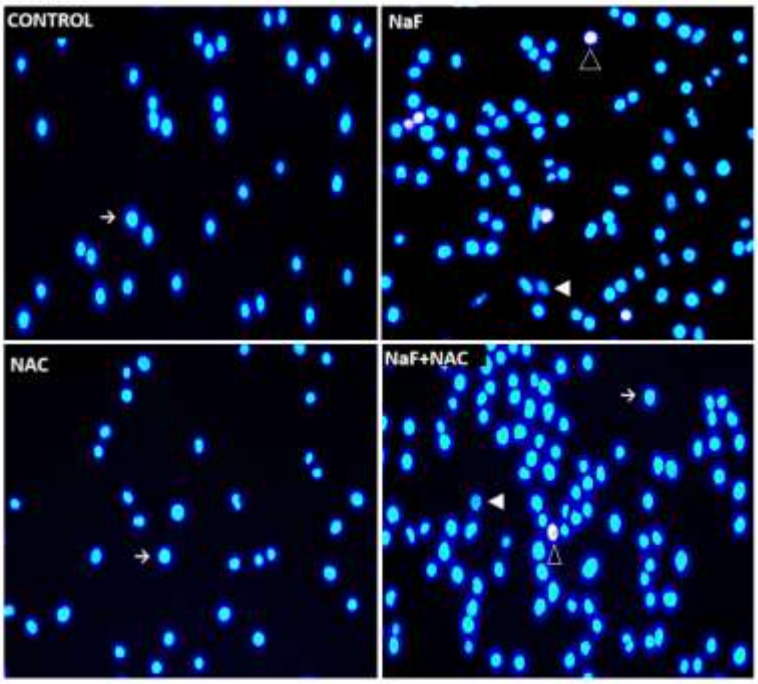


Figure 4. Fluorescence microscope image showing the effects on live, apoptotic and dead cells in TM3 Leydig cells after 24 hours in control, NaF and Nac groups (□: viable cells,□: Apoptotic cells,□: Dead cells).

DISCUSSION

Studies have shown that high levels of fluoride exposure can cause toxic effects on different systems of the body (Fishta et al., 2024; Masnaoui, 2024; Taher et al., 2024). Analysis of clinical research and animal tests clearly demonstrates that exposure to fluoride leads to both structural and functional abnormalities in spermatozoa, resulting in reduced sperm count and infertility. These adverse effects have a detrimental impact on male reproductive functions (Chaithra et al., 2020; He et al., 2023). Based on these studies, safe doses of sodium fluoride (NaF) can be determined, and its use can be limited. The role of Nac, which has strong antioxidant properties, in reducing NaF-induced damage in Leydig cells was investigated for the first time in this study.

Several studies have shown that NaF enhances cytotoxicity (Liu et al., 2008; Kim et al., 2015; Kumar et al., 2018; Pal et al., 2021). The LDH test is utilized to ascertain the level of activity shown by the lactate dehydrogenase enzyme that is discharged into the cytoplasm as a result of cellular damage and death (Castiglione et al., 2024). In a study conducted with human liver cells (L-02), as a result of 48-hour exposure to sodium fluoride (NaF) at different concentrations (100 μg/mL, 200 μg/mL, and 400 μg/mL), a significant increase in LDH level was observed depending on the increasing NaF concentration (Guo et al., 2024). In a study with microglial cells (BV-2), exposure to 0, 0.5, 1, and 2 mmol/L NaF was performed for 24 hours. The results showed that cell membrane integrity was damaged dose-dependently after fluoride exposure and caused an increase in LDH levels compared to the control (Zhang et al., 2024). In a study aimed at illustrating the therapeutic potential of Nac, the human dermal fibroblasts

(HDFs) were grown in a normal medium (5.5 mM glucose) for 72 h, and the experimental groups were grown in medium containing 25-, 50-, and 75-mM glucose concentrations. After 1 mM Nac treatment was applied to cells exposed to 50 mM and 75 mM glucose, a significant increase in LDH was observed in the cultured HDFs, while Nac was observed to attenuate these effects (Rashnavadi et al., 2022). Similar to previous studies, our *in vitro* study using Leydig cells showed that 50 ppm fluoride increased cytotoxicity, while Nac provided significant protection against fluoride toxicity.

Research has shown that the accumulation of reactive oxygen species within cells leads to oxidative stress, which damages important cellular components like proteins, DNA, and lipids. This damage can ultimately result in cell death, a process known as apoptosis (Redza-Dutordoir & Averill-Bates, 2016; Galadari et al., 2017; Cadenas, 2018). Several studies have extensively established that fluoride acts through various apoptotic pathways (Zhang et al., 2016; Ni et al., 2018; Song et al., 2021; Zhou et al., 2022). A study conducted using human lung epithelial cells in a laboratory setting demonstrated that a concentration of 2 mM NaF resulted in an elevation of cell mortality through the activation of caspases after a 24-hour period (Song et al., 2021). A study conducted on cementoblast cells found that treatment to NaF at concentrations of 5 mM and 10 mM resulted in the cells displaying characteristics of apoptosis, such as nuclear condensation and fragmentation, as compared to the control group (Ni et al., 2018). During an experiment involving rat fibroblast cells, researchers noted alterations in the expression of apoptosis-related genes, such as Bax and Bcl2, following a 24-hour exposure to a fluoride concentration of 1 mM. Zhou et al. (2022) found that exposure to fluoride led to a significant drop in Bcl2 expression, while Bax expression was observed to increase. A further investigation explored the beneficial impact of Nac on countering the harmful consequences of 2-ethylhexyldiphenyl phosphate. The study revealed that Nac effectively inhibited cell death (Wang et al., 2024). In parallel with the previous studies, our findings revealed that 50 ppm fluoride triggered apoptosis, while 1 mM Nac inhibited apoptosis.

In conclusion, this study showed that NaF induced cytotoxicity and apoptosis in Leydig cells. Nac suppressed cytotoxicity and reduced apoptotic cell death against the damage caused by NaF. As a result, this study revealed that Nac inhibited cellular damage by preventing apoptosis.

FUNDING

This study was supported by Istanbul University Scientific Research Projects (Project No. 40296).

REFERENCES

Ashoori, R., Samaei, M. R., Yousefinejad, S., Azhdarpoor, A., Emadi, Z., Mohammadpour, A., ... & Khaneghah, A. M. (2022). Simultaneous removal of fluoride and nitrate from synthetic aqueous solution and groundwater by the electrochemical process using non-coated and coated anode electrodes: A human health risk study. *Environmental Research*, 214, 113938.

Banerjee, A., & Roychoudhury, A. (2019). Fluorine: a biohazardous agent for plants and phytoremediation strategies for its removal from the environment. *Biologia Plantarum*, *63*(1), 104-112.

Chaithra, B., Sarjan, H. N., & Shivabasavaiah. (2018). Dose and time-dependent effects of sodium fluoride on sperm motility: An in vitro study. *Toxicology and Industrial Health*, *34*(12), 813-818.

Chaithra, B., Sarjan, H. N., & Shivabasavaiah. (2020). A comparative analysis of fluoride-contaminated groundwater and sodium fluoride-induced reproductive toxicity and its reversibility in male rats. *Biological Trace Element Research*, *197*, 507-521.

Chaudhari, S., Parmar, H., & Samnani, P. (2024). Human Nutritional Condition and Dental Fluorosis in Populations with Varying Concentrations of Fluoride in Their Water Sources. In *Advanced Treatment Technologies for Fluoride Removal in Water: Water Purification* (pp. 251-269). Cham: Springer Nature Switzerland.

Fishta, A., Thakur, R., Sharma, K. C., Thakur, N., & Patial, B. (2024). Effects of Fluoride Toxicity on Female Reproductive System of Mammals: A Meta-Analysis. *Biological Trace Element Research*, 1-24.

Galadari, S., Rahman, A., Pallichankandy, S., & Thayyullathil, F. (2017). Reactive oxygen species and cancer paradox: to promote or to suppress?. *Free Radical Biology and Medicine*, 104, 144-164.

Guo, W. Y., Lu, D. Y., Guan, Z. Z., Zheng, L., Chen, S. S., & Liu, T. (2024). Periploca forrestii Schltr. ameliorate liver injury caused by fluorosis in rat. *Ecotoxicology and Environmental Safety*, *270*, 115813.

He, J., Mu, Y., Liu, M., Che, B. W., Zhang, W. J., Chen, K. H., & Tang, K. F. (2023). Glutathione S-transferase genetic polymorphisms and fluoride-induced reproductive toxicity in men with idiopathic infertility. *Asian Journal of Andrology*, *25*(3), 404-409.

Herzenberg, L. A. (2019). History of N-acetylcysteine. In R. E. Frye & M.Berk (Eds.), The therapeutic use of N-acetylcysteine (NAC) in medicine(pp. 3–15). Singapore: Springer.

Kim, J., Kwon, W. S., Rahman, M. S., Lee, J. S., Yoon, S. J., Park, Y. J., ... & Pang, M. G. (2015). Effect of sodium fluoride on male mouse fertility. *Andrology*, *3*(3), 544-551.

Kumar, P., Nagarajan, A., & Uchil, P. D. (2018). Analysis of cell viability by the lactate dehydrogenase assay. Cold Spring Harbor Protocols, 2018(6), pdb-prot095497.

Liang, C., Gao, Y., He, Y., Han, Y., Manthari, R. K., Tikka, C., ... & Zhang, J. (2020). Fluoride induced mitochondrial impairment and PINK1-mediated mitophagy in Leydig cells of mice: *in vivo* and *in vitro* studies. *Environmental Pollution*, *256*, 113438.

Liu, H., Niu, R., Wang, J. M., He, Y., Wang, J. D., & China, S. (2008). Changes caused by fluoride and lead in energy metabolic enzyme activities in the reproductive system of male offspring rats. *Fluoride*, *41*(3), 184-191.

Long H, Jin Y, Lin M, Sun Y, Zhang L, Clinch CA (2003). Fluoride toxicity in the male reproductive system. *Fluoride,* 42(4):260–276

Masnaoui, L. A. K., Bouchab, H., Rahim, A., El Kebbaj, R., & Essamadi, A. (2024). Association between Fluoride Toxicity, Oxidative Stress, and Pregnancy Complications in Women Living in Fluorosis Areas. *Baghdad Science Journal*, *21*(4), 1204-1204.

Mikkonen, H. G., van de Graaff, R., Mikkonen, A. T., Clarke, B. O., Dasika, R., Wallis, C. J., & Reichman, S. M. (2018). Environmental and anthropogenic influences on ambient background concentrations of fluoride in soil. *Environmental Pollution*, *242*, 1838-1849.

Ni, J., Li, Y., Zhang, W., Shu, R., & Zhong, Z. (2018). Sodium fluoride causes oxidative stress and apoptosis in cementoblasts. *Chemico-Biological Interactions*, 294, 34-39.

Oncü M, Kocak A, Karaoz E, Darici H, Savik E, Gultekin F (2007) Effect of long-term fluoride exposure on lipid peroxidation and histology of testes in first- and second-generation rats. *Biological Trace Element Research,* 118:260–268

Pal, P., & Mukhopadhyay, P. K. (2021). Fluoride induced testicular toxicities in adult Wistar rats. *Toxicology mechanisms and methods*, *31*(5), 383-392.

Radovanović, J., Antonijević, B., Ćurčić, M., Baralić, K., Kolarević, S., Bulat, Z., ... & Mandinić, Z. (2022). Fluoride subacute testicular toxicity in Wistar rats: Benchmark dose analysis for the redox parameters, essential elements and DNA damage. *Environmental Pollution*, *314*, 120321.

Rahmani, S., & Rezaei, M. (2020). Toxicity of fluoride on isolated rat liver mitochondria. *Journal of Fluorine Chemistry*, *239*, 109636.

Rashnavadi, M. H., Tahmasebi, Z., Piravar, Z., & Gholi, A. M. (2022). N-Acetyl-L-cysteine effects on oxidative stress-induced high glucose-cultured human dermal fibroblasts. *Gene Reports*, *26*, 101522.

Redza-Dutordoir, M., & Averill-Bates, D. A. (2016). Activation of apoptosis signalling pathways by reactive oxygen species. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1863(12), 2977-2992.

Schwalfenberg, G. K. (2021). N-acetylcysteine: a review of clinical usefulness (an old drug with new tricks). *Journal of Nutrition and Metabolism*, 2021(1), 9949453.

Song, C., Shi, D., Chang, K., Li, X., Dong, Q., Ma, X., ... & Wang, J. (2021). Sodium fluoride activates the extrinsic apoptosis via regulating NOX4/ROS-mediated p53/DR5 signaling pathway in lung cells both *in vitro* and *in vivo*. *Free Radical Biology and Medicine*, 169, 137-148.

Su, H., Kang, W., Kang, N., Liu, J., & Li, Z. (2021). Hydrogeochemistry and health hazards of fluoride-enriched groundwater in the Tarim Basin, China. *Environmental Research*, *200*, 111476.

Taher, M. K., Momoli, F., Go, J., Hagiwara, S., Ramoju, S., Hu, X., ... & Krewski, D. (2024). Systematic review of epidemiological and toxicological evidence on health effects of fluoride in drinking water. *Critical Reviews in Toxicology*, *54*(1), 2-34.

Veneri, F., Vinceti, M., Generali, L., Giannone, M. E., Mazzoleni, E., Birnbaum, L. S., ... & Filippini, T. (2023). Fluoride exposure and cognitive neurodevelopment: Systematic review and dose-response meta-analysis. *Environmental Research*, *221*, 115239.

Wang, J., Zhang, Y., Zhang, H., Zhang, K., Zhang, Z., & Li, J. (2009). Toxic effects of fluoride on reproductive ability in male rats: sperm motility, oxidative stress, cell cycle, and testicular apoptosis. *Fluoride*, *42*(3), 174.

Wang, Y., Guo, A. L., Xu, Y., Xu, X., Yang, L., Yang, Y., & Chao, L. (2024). EHDPP induces proliferation inhibition and apoptosis to spermatocyte: Insights from transcriptomic and metabolomic profiles. *Ecotoxicology and Environmental Safety*, 284, 116878.

Zhang, Q., Li, T., Shi, R., Qi, R., Hao, X., & Ma, B. (2024). Fluoride promotes the secretion of inflammatory factors in microglia through NLRP3/Caspase-1/GSDMD pathway. *Environmental Science and Pollution Research*, *31*(13), 19844-19855.

Zhang, S., Niu, Q., Gao, H., Ma, R., Lei, R., Zhang, C., ... & Wang, A. (2016). Excessive apoptosis and defective autophagy contribute to developmental testicular toxicity induced by fluoride. *Environmental Pollution*, *212*, 97-104.

Zhang, Y., Dong, F., Wang, Z., Xu, B., Zhang, T., Wang, Q., & Lin, Q. (2023). Fluoride exposure provokes mitochondria-mediated apoptosis and increases mitophagy in osteocytes via increasing ROS production. *Biological Trace Element Research*, *201*(8), 3994-4007.

Zhou, Z., Cui, Y., Zhang, X., & Zhang, Y. (2021). The role of N-acetyl-cysteine (NAC) orally daily on the sperm parameters and serum hormones in idiopathic infertile men: A systematic review and meta-analysis of randomised controlled trials. *Andrologia*, *53*(2), e13953.

Zhou, W., Luo, W., Liu, D., Canavese, F., Li, L., & Zhao, Q. (2022). Fluoride increases the susceptibility of developmental dysplasia of the hip via increasing capsular laxity triggered by cell apoptosis and oxidative stress *in vivo* and in vitro. *Ecotoxicology and Environmental Safety*, 234, 113408.

CONVERSION OF BREAD WASTE TO VALUE-ADDED PRODUCTS

Göksel TIRPANCI SİVRİ[1], Ahmet Şükrü DEMİRCİ[1]

[1] Tekirdağ Namık Kemal University, Agricultural Faculty, Food Engineering Department, Tekirdağ, Türkiye
Corresponding author e-mail: ademirci@nku.edu.tr

ABSTRACT

In many countries, waste bread is one of the most important components of food waste. Due to short shelf life and overproduction, ~10% of bread (900,000 tonnes) is wasted globally along the supply chain from producer to consumer consumption. This leads to a large amount of unused bread in supermarkets and households. However, waste bread has a high potential to be utilized as a renewable raw material. Bread waste is a potential biosource for the production of fermentable sugars. Starchy food waste is easily degradable and therefore a good alternative biomass source. Fermentation is the most researched strategy for the recycling of bakery waste. The saccharification and hydrolysis of starch in bread with the help of enzymes causes the release of simple sugars. These simple sugars can then be utilized by microorganisms and converted into valuable products. Ethanol, hydrogen, lactic acid, 2,3-butanediol, paramylon, and xanthan gum can be produced by microorganisms through further fermentation of the hydrolysate following hydrolysis. In this review, the latest advancements in bread waste recycling are discussed, the potential for producing novel chemicals is explored, and the efficiency of converting bread waste into fermentable sugars for new product development is evaluated.

Key words: Waste bread, biomass, food waste

INTRODUCTION

In our world where 870 million people are malnourished annually, food waste has reached serious dimensions. According to FAO, it is estimated that 1.3 billion tonnes of food with an annual economic value of 1 trillion dollars is wasted. FAO data show that the amount wasted or lost constitutes one third of the world's food production (FAO, 2013). Nowadays, the large number of supermarkets and fast food chains, especially in developed countries, make it easier for people to access food and waste more food. While the growing number of supermarkets and fast food chains in developed countries has made food more accessible, it has also contributed to a significant increase in food waste. Moreover, the increase in food diversity and the appeal of attractive packaging encourage people to purchase more than they need, further exacerbating wastage. As a result, large quantities of various food types are wasted globally, with bread being one of the most significant contributors to this waste (Demirci et al. 2017).

Bread has been the most fundamental food source for people worldwide and the most produced and consumed food product throughout history. Waste occurs when bread is not used for its intended purpose, such as not being consumed by humans or being discarded. Even when bread is given to animals rather than being eaten, it is still considered waste. This is because the resources—such as labor, energy, and income—invested in producing flour and baking bread are effectively lost when the bread is not consumed by people. Therefore, the use of bread instead of grain products that can be used directly as animal feed is considered wasteful in economic terms. In Turkey, 95 million pieces of bread are consumed daily and 35 billion pieces of bread are consumed annually. However, 1,500 tonnes of bread is wasted per day and 542 thousand tonnes per year (TMO, 2013). In 2019, Dutch households wasted over 7.3kg of bread and pastries. This is 20% of the bread produced in the same year (van Gelder, 2020a, 2020b). In Sweden, 80,410 tonnes of bread are wasted every year, which is 8.1 kg per capita per year (Brancoli et al.,2019). It is estimated that a person in Spain consumes about 46-58 kg of bread per year. Bread waste (BW) arises in industrial factories and is removed due to poor wheat quality, errors in baking technology, storage, transport systems or new market demands (commercial crustless bread). According to statistical data, 30% of the total bread produced in Spain (660 million kg out of a production of 2200 million kg) is food waste (Sanchez et al 2014). In the United Kingdom (UK), the bread and sandwich bread sector is one of the largest food industries. The amount of bread waste is estimated to reach approximately 1.2 million tonnes per year (Melikoğlu, 2008). The most important factor causing bread waste is stale or mouldy bread before it can be consumed as a result of inappropriate storage conditions. One of the reasons for bread waste is the lack of knowledge about the methods of utilizing stale bread. According to the Tesco supermarket chain, around 10% of baked goods ended up in the bin in the UK in 2021–2022 (Bedford, 2022). Some of the bread that is not consumed but returned to bakeries is processed into breadcrumbs or animal feed, but a large proportion is unsuitable for such reprocessing due to mould contamination, high degree of staling and other reasons.

Bread waste and by-products are generated throughout the stages of processing, manufacturing, and retail. Various factors influence the quality of bakery products. Many physical and chemical changes occur during the storage of bread with short shelf life. Changes such as taste, aroma, water absorption capacity, crystallinity, opacity, soluble starch content and tightening of the bread cause the bread to spoil. Spoilt bread loses its sensory properties. Although bread is still healthy and nutritious, these changes cause a negative perception in the consumer. As a result, large quantities of bread are thrown away and cause great economic loss. Therefore, waste bread is a very important waste food problem (Melikoğlu and Webb, 2013). BW, being one of the most commonly discarded foods, has the potential to serve as a renewable resource. Fermentation is the most frequently discussed method for recycling bread waste. This waste is rich in starch, proteins, and nutrients, making it ideal for fermentation processes (Mihajlovski et al., 2020). In addition, there are other options proposed by researchers for recycling bakery waste. The aim of this review is to discuss the latest developments in the recycling of bread waste, to investigate the potential to produce new chemicals and to determine the efficiency of using bread waste to produce sugar, which is used to make a new product through fermentation.

Bread waste composition and enzymatic hydrolysis

The nutritional content of bread varies according to the type of bread, but in general 100 g of white bread contains about 50 g of carbohydrates (47 g in the form of starch), 37 g of water and about 8 g of protein. This composition makes bread a perfect and almost complete source of nutrition for many microorganisms as well as for its future consumers (Melikoğlu and Webb, 2013). Bread contains high amounts of starch, which can be easily hydrolysed to monomeric sugars using enzymes. Bread contains 500-750 g kg-1 starch and 3-50 g kg-1 simple sugars (Dewettinck et al. 2008). It also contains 100-150 g kg-1 protein (Thomas and Ingledew 1990). The composition of one slice of bread is shown in Table 1.

Table 1. Composition of a single slice of waste bread

Component	Weight (g)
Water	28.67
Starch	45.34
Nitrogen (N)	1.61
Protein (N x 5.7)	9.18
Phosphorus	0.10
Ash	2.26

Nutrients in foods are in the form of macromolecules such as starch and proteins. To be utilized by microorganisms, these higher molecules must be broken down into usable forms such as sugars and amino acids. Usually, this process is carried out by enzymatic hydrolysis using amylase and protease enzymes (Sarkar et al. 2012). Starch is the main component of the dry weight of bread. However, starch is a substrate for alcohol and other fermentations in industry and waste bread can be utilized as a fermentation nutrient medium for the production of various products. For the starch in bread to be used in the production of fermentation products, it must be converted into fermentable simple sugars. The conversion of starch into individual glucose units involves the hydrolysis of these α-(1 – 4) and α-(1 – 6) linkages, which can be made possible by the action of acid (H_2SO_4/HCl) or enzymes (α-amylase and glucoamylase) (Bello-Perez et al., 2020). Compared to acid or alkaline hydrolysis, enzymatic hydrolysis offers significant advantages, such as lower toxicity, cost-effectiveness, and reduced corrosion. Additionally, enzymatic hydrolysis does not produce inhibitory by-products. As a result of acid hydrolysis, various by-products such as acetic acid, furfural and 5 hydroxymethylfurfural are formed. These products inhibit the growth of microorganisms. Therefore, hydrolysates to be used for fermentation should be subjected to detoxification (Sarkar et al. 2012).

Traditionally, acid hydrolysis has been employed to produce soluble starch from insoluble starch granules by treating the starch with high concentrations of acid at room temperature for an extended period (Höfer, 2015). It was recognized that acids like HCl and H_2SO_4 break glucosidic linkages and modify the physical and chemical properties of native starch.

The enzymatic hydrolysis of starch to fermentable sugars has a typical 2-step method. In the first step, the gelatinized starch solution is liquefied with the temperature-stable enzyme α-amylase (EC 3.2.1.1) to reduce its viscosity and break the α-1.4 glycosidic bonds in the middle of the amylose and amylopectin chain to form short-chain dextrins. In the second step of starch hydrolysis (saccharification), dextrins are saccharified with the enzyme glucoamylase (amyloglucosidase) (EC 3.2.1.3) to yield monomer sugars (glucose) (Demirci et al., 2016). Generally, supporting enzymes such as protease, cellulase, pullulanase etc. are also used to increase the amount of fermentable sugars, to reduce the viscosity of the pellet and to obtain free amino nitrogen that the microorganism can use as food during the fermentation phase (Sapinska et al. 2013). Demirci and colleagues investigated the enzymatic hydrolysis of bread waste during the liquefaction and saccharification stages, using different bread waste loadings. They achieved a maximum glucose conversion yield of 86%, which corresponds to 99% of the theoretical maximum. This was accomplished under optimal conditions of 0.05 g/g substrate loading, 0.03 KNU/g α-amylase, and 3.6 AGU/g glucoamylase (Demirci et al., 2017).

Waste bread utilisation by fermentation

As mentioned earlier, BW is a rich source of high-quality fermentable food-grade sugars, along with other nutrients, making food or bread waste an appealing substrate for fermentation processes. The simple sugars obtained are fermented to produce new products. This type of fermentation is used in biorefineries, in the production of bioethanol, fuels and chemicals, in the brewing industry, etc. (Nigam, 2013).

Ethanol Production

Raw materials that contain high levels of sugar or starch-cellulose, which can be converted into simple sugars, can be used in ethanol production. Food price increases and ethical problems caused by the use of agricultural products in ethanol production and the solution of technological difficulties in the conversion of lignocellulosic agricultural wastes reveal the necessity of discovering new raw materials and using them for this purpose. The utilisation of food wastes in this direction is important and studies on these issues have been increasing in recent years. Studies on the utilisation of waste bread in ethanol production have shown that bread can be a highly efficient ethanol fermentation feedstock.

Ebrahimi et al. (2008) investigated alcohol production by separate hydrolysis and fermentation of waste bread containing 350 g kg -1 raw material and obtained alcohol yield as 350 g/kg bread dry matter. Pietrzak and Kawa-Rygielska (2014) investigated the ethanol conversion of waste wheat-rye bread directly from starch using Granular Starch Hydrolyzing Enzymes (GSHE) and the ethanol yield was determined as 334.81 g kg sugar. Mihajlovski et al. (2020) utilized bread waste to produce bioethanol by employing crude hydrolytic enzymes from the Hymenobacter sp. CKS3 strain. The hydrolysate primarily consists of dextrins, maltotriose, maltose, and glucose. Bread waste hydrolysate contains 19.89 g of reducing sugars per liter, which, when combined with waste baker's yeast, produces 1.73% ethanol (Mihajlovski et al., 2020). More recently, Narisetty et al. (2022) conducted an in-depth study on BW hydrolysis followed by ethanol fermentation. BW was saccharified using both acidic and enzymatic hydrolysis, with both methods optimized for solid and acid/enzyme loading to maximize productivity. The acidic and enzymatic hydrolysis of BW achieved maximum glucose releases of 75.0 g/L and 97.9 g/L, respectively, at a solid loading of 20% w/v, corresponding to 73.5% and 95.9% of the maximum theoretical yield. The resulting hydrolysate was then fermented into ethanol. The fed-batch fermentation using glucose-rich acidic and enzymatic hydrolysates resulted in ethanol titers of 106.9, and 114.9 g/L with a conversion yield and productivity of 0.47, and 0.49 g/g, and 3.0, and 3.2 g/L.h, respectively. Studies have shown that waste bread is a highly efficient and unique resource that can be used in ethanol production. The high carbohydrate content of bread, which can be converted into fermentable sugars, shows that it can be a good raw material for bioethanol production.

Lactic Acid Production

Cox et al. (2022) employed *B. coagulans* DSM1 for lactic acid fermentation of bread waste under non-sterile conditions. The study found that 419.1 g of lactic acid could be produced from 1 kg of waste bread, with 155.4 g of lactic acid being generated from 182.8 g of glucose derived from 370.8 g of bread waste. In a recent study by Olszewska-Widdrat et al. (2020), lactic acid (LA) was produced from sugar bread (SB) using *B. coagulans*. The SB hydrolysate, obtained after liquefaction and saccharification, contained 76.9 g/L of glucose, 9.98 g/L of fructose, and 38.5 g/L of disaccharides. Batch fermentation of this SB hydrolysate by *B. coagulans* resulted in LA titers of 80.0 g/L after 30 hours. Sadaf et al. (2021) utilized various strains of *Lactobacillus paracasei* (SKL-9, SKL-11, and SKL-21) to produce lactic acid from 598 mg of reducing sugars. The results were as follows: SKL-9 produced 53 mg of lactic acid per gram of bread waste, SKL-11 produced 56 mg per gram, and SKL-21 produced 54 mg per gram, which corresponded to 26, 28, and 27 g/L, respectively.

Biohydrogen production

Doi et al. (2009) carried out biohydrogen production by anaerobic fermentation using rhizosphere microflora and obtained a yield of 1.3 mol H_2 mol- 1 glucose. According to Han et al. (2016), 1g of waste bread could generate 0.332 g of glucose which could be further utilized to produce 109.5 ml of hydrogen (1.6 mol H_2 mol^{-1}glucose). Adessi et al. (2018) used a sequential system composed of lactic fermentation and photofermentation, yielding 3.1 mol H_2 mol^{-1} glucose.

2,3-Butanediol production

2,3-Butanediol, also known as 2,3-butylene glycol or dimethyl ethylene glycol, is a chiral compound that can exist as either a colorless and odorless liquid or a crystalline solid. It can be produced economically through the bioconversion of natural resources. 2,3-Butanediol can be converted into 1,3-butadiene, a key component in synthetic rubber production. Its derivatives are utilized in various applications, including as freezing agents, solvents, flavorings, and in the production of plastics. 2,3-Butanediol is used in the food, chemical, pharmaceutical, petrochemical and aerospace industries (Syu, 2001). Maina et al. (2021) evaluated the production of D-2,3-butanediol by *Bacillus amyloliquefaciens* using bakery waste hydrolysates. The highest yield of D-2,3-butanediol achieved from bread waste was 55.2 g/L. Narisetty et al. (2022) utilized *Enterobacter ludwigii* to produce 2,3-butanediol from bread waste. Through acidic and enzymatic hydrolysis, they achieved a glucose yield of 330–530 g per kilogram of bread waste. This glucose was then used by *E. ludwigii* for the production of 2,3-butanediol. The study demonstrated that 150–200 g of 2,3-butanediol can be produced from 1 kg of bread waste that has been saccharified by either acid or enzymatic pre-treatment.

Xanthan gum biosynthesis

Xanthan gum is a non-toxic, water-soluble, biodegradable, biocompatible, and thermally stable substance that remains stable under both acidic and alkaline conditions (Kumar et al., 2018). It is commonly used as a thickener and stabilizer for suspensions and emulsions in the food industry (Nasrabadi et al., 2016). Demirci et al. (2019) demonstrated the successful use of bread waste hydrolysate as a biological source for xanthan gum production. Bread waste was cut into small pieces, pulverized in a laboratory mill, and enzymatically hydrolyzed. The resulting hydrolyzed bread solution was diluted with sterile distilled water to achieve sugar concentrations of 40, 80, and 120 g/L, and then treated with *Xanthomonas campestris* NRRL B-1459, *Xanthomonas axonopodis*, *Xanthomonas hortorum*, and *Xanthomonas axonopodis*. The highest xanthan gum production levels and conversion yields were 12.5 g/L (0.166 g xanthan per gram of sugar) for *X. hortorum*, 10.3 g/L (0.121 g xanthan per gram of sugar) for *X. axonopodis*, 14.3 g/L (0.149 g xanthan per gram of sugar) for *X. axonopodis*, and 12.9 g/L (0.155 g xanthan per gram of sugar) for *X. campestris*. This study indicates that hydrolysates from bread waste could be a promising and cost-effective carbon source for xanthan gum production.

Other fermented products

There are other studies related to the biocycling of waste bread. In other studies where bread was used as raw material; the production of flavour compounds with *Geotrichum candidum* (Daigle et al. 1999), production of amylase and protease enzymes with *Aspergillus awamori* solid phase fermentation (Melikoğlu et al. 2013a; Melikoğlu et al. 2013b), succinic acid production with *Actinobacillus succinogenes* (Leung et al. 2012).

CONCLUSIONS

Bread is an excellent raw material for biotechnological processes as it contains carbon, nitrogen, major and minor nutrients in a balanced way. BW, one of the most commonly wasted foods, can be repurposed as a renewable resource through modern utilisation methods and technologies. Fermentation is a widely discussed strategy for recycling bread waste. It serves as an excellent feedstock for microorganisms such as bacteria, fungi, and yeasts. These microorganisms produce enzymes that facilitate the saccharification and hydrolysis of polysaccharides in the bread waste. Previous studies have shown that waste bread is a highly efficient, unique resource that can be used in the production of high value-added products. This approach will also have an important result in terms of preventing environmental pollution by using wastes in an environmentally friendly manner.

REFERENCES

Adessi, A., Venturi, M., Candeliere, F., Galli, V., Granchi, L., & De Philippis, R. (2018). Bread wastes to energy: Sequential lactic and photo-fermentation for hydrogen production. *International Journal of Hydrogen Energy, 43*(20), 9569–9576.

Bedford, E. (2022). Share of Tesco store food waste in the United Kingdom (UK) in 2021/ 22, by category breakdown. *Statista*.

Bello-Perez, L.A., Flores-Silva, P.C., Agama-Acevedo, E., Tovar, J., 2020. Starch digestibility: past, present, and future. J Sci Food Agric.

Brancoli, P., Lundin, M., Bolton, K., Eriksson, M., 2019. Bread loss rates at the supplierretailer interface – Analysis of risk factors to support waste prevention measures. Resour Conserv Recycl 147, 128–136.

Cox, R., Narisetty, V., Nagarajan, S., Agrawal, D., Ranade, V.V., Salonitis, K., Venus, J., Kumar, V., 2022. High-Level fermentative production of Lactic acid from bread waste under Non-sterile conditions with a circular biorefining approach and zero waste discharge. Fuel 313.

Daigle, P., Gelinas, P., Leblanc, D., & Morin, A. (1999). Production of aroma compounds by Geotrichum candidum on waste bread crumbs. Food Microbiology, 16, 517–522.

Demirci, A.Ş, Palabıyık, I., Gümüs, T., Ozalp, ¨ S¸., 2017. Waste Bread as a Biomass Source: Optimization of Enzymatic Hydrolysis and Relation between Rheological Behavior and Glucose Yield. Waste Biomass Valorization 8, 775–782.

Demirci, A. S., Palabiyik, I., Apaydın, D., Mirik, M., & Gumus, T. (2019). Xanthan gum biosynthesis using *Xanthomonas* isolates from waste bread: Process optimization and fermentation kinetics. *Lebensmittel-Wissenschaft und -Technologie, 101*, 40–47.

Dewettinck K, Van Bockstaele F, Küchne B, Van de Walle D, Courtens TM, Gellynck X. Nutritional value of bread: influence of processing, food interaction and consumer perception. J Cereal Sci 2008;48:243–57.

Doi, T., Matsumoto, H., Abe, J., & Morita, S. (2009). Feasibility study on the application of rhizosphere microflora of rice for the biohydrogen production from wasted bread. *International Journal of Hydrogen Energy, 34*(4), 1735–1743.
Ebrahimi, F., Khanahmadi, M., Roodpeyma, S., & Taherzadeh, M. (2008). Ethanol production from bread residues. *Biomass and Bioenergy, 32*(4), 333–337.
FAO. 2013. Food wastage footprint. Impacts on natural resources. Summary Report. Natural Resources Management and Environment Department. http://www.fao.org/docrep/018/i3347e/i3347e.pdf.
van Gelder, K. (2020a). *Most frequently wasted food products in The Netherlands 2019*. Statista. https://www.statista.com/statistics/733120/most-frequently-wasted-foodproducts-in-the-netherlands/.
van Gelder, K. (2020b). *Reasons to throw away bread and pastries in Flanders (Belgium) 2017-2018. Statista*.
Han, W., Huang, J., Zhao, H., & Li, Y. (2016). Continuous biohydrogen production from waste bread by anaerobic sludge. *Bioresource Technology, 212*, 1–5.
Höfer, ¨ R., 2015. Sugar-and Starch-Based Biorefineries, Industrial Biorefineries and White Biotechnology.
Kumar, V., Longhurst, P., 2018. Recycling of food waste into chemical building blocks. Curr Opin Green Sustain Chem 13, 118–122.
Leung, C.C.J., Cheung, A.S.Y., Zhang, A.Y.Z., Lam, K.F., Lin, C.S.K., 2012. Utilisation of waste bread for fermentative succinic acid production. Biochem Eng J 65, 10–15.
Maina, S., Prabhu, A.A., Vivek, N., Vlysidis, A., Koutinas, A., Kumar, V., 2021. Prospects on bio-based 2,3-butanediol and acetoin production: Recent progress and advances. Biotechnol Adv 107783.
Melikoglu, M. Production of Sustainable Alternatives to Petrochemicals and Fuels Using Waste Bread as a Raw Material, School of Chemical Engineering and Analytical Science, The University of Manchester, Manchester, United Kingdom, 2008 (Ph.D. Thesis).
Melikoglu, M., Webb, C. Use of waste bread to produce fermentation products. In: Kosseva, M., Webb, C. (eds.) Food Industry Wastes: Assessment and Recuperation of Commodities. Academic Press, London (2013).
Melikoglu M, Lin CSK, Webb C (2013a) Kinetic studies on the multi-enzyme solution produced via solid state fermentation of waste bread by Aspergillus awamori. Biochem Eng J 80:76–82
Melikoglu M, Lin CSK, Webb C (2013b) Stepwise optimisation of enzyme production in solid state fermentation of waste bread pieces. Food Bioprod Process 91(4):638–646.
Mihajlovski, K., Rajili´c-Stojanovi´c, M., & Dimitrijevi´c-Brankovi´c, S. (2020). Enzymatic hydrolysis of waste bread by newly isolated *Hymenobacter sp.* CKS3: Statistical optimization and bioethanol production. *Renewable Energy, 152*, 627–633.
Narisetty, V., Nagarajan, S., Gadkari, S., Ranade, V. v., Zhang, J., Patchigolla, K., Bhatnagar, A., Kumar Awasthi, M., Pandey, A., Kumar, V., 2022. Process optimization for recycling of bread waste into bioethanol and biomethane: A circular economy approach.
Nasrabadi, M. N., Goli, S. A. H., & Nasirpour, A. (2016). Stability assessment of conjugated linoleic acid (CLA) oil-in-water beverage emulsion formulated with acacia and xanthan gums. *Food Chemistry, 199*, 258–264.
Nigam, P. (2013). Microbial enzymes with special characteristics for biotechnological applications. *Biomolecules, 3*(4), 597–611.
Olszewska-Widdrat, A., Alexandri, M., Lopez-G ´ omez, ´ J.P., Schneider, R., Venus, J., 2020. Batch and continuous lactic acid fermentation based on a multi-substrate approach. Microorganisms 8, 1–14.

Pietrzak, W., & Kawa-Rygielska, J. (2014). Ethanol fermentation of waste bread using granular starch hydrolyzing enzyme: Effect of raw material pre-treatment. *Fuel, 134*, 250–256.
Sadaf, A., Kumar, S., Nain, L., & Khare, S. K. (2021). Bread waste to lactic acid: Applicability of simultaneous saccharification and solid state fermentation. *Biocatalysis and Agricultural Biotechnology, 32*, Article 101934.
Sánchez,C., Serrano, L., Llano-Ponte, R., Labidi, J. Bread residues conversion into lactic acid by alkaline hydrothermal treatments. Chemical Engineering Journal 250 (2014) 326–330.
Sapinska E, Balcerek M, Pielech-Przybylska K. Alcoholic fermentation of highgravity corn mashes with the addition of supportive enzymes. J Chem Technol Biotechnol 2013;88:2152–8.
Sarkar, P., Valacchi, G., & Duary, R. K. (2022). Proteome composition and profiling of bioactive peptides of edible Antheraea assamensis pupae by sequential enzymatic digestion and kinetic modeling of in vitro gastrointestinal digestion. *European Food Research and Technology*, 1-23.
Syu, M.-J. (2001). Biological production of 2,3-butanediol. *Applied Microbiology and Biotechnology*, *55*(1), 10–18.
Thomas KC, Ingledew WM. Fuel alcohol production: effects of free amino nitrogen on fermentation of very-high-gravity wheat mashes. Appl Environ Microbiol 1990;56:2046–50.
TMO. 2013. Ekmek İsrafını Önleme Kampanyası Toplantısı,http://www.tmo.gov.tr/

EXAMINING THE EFFECT OF SUSTAINABILITY-FOCUSED CERTIFICATION IN PALM OIL PRODUCTION ON PRODUCER AND CONSUMER AWARENESS

Zahide Dündar[1], *Ruhan Aşkın Uzel*[2], *Onur Özdikicierler*[3]
[1]Ege University, Graduate School of Natural and Applied Sciences, Food Engineering Department, İzmir, Türkiye
[2]Yaşar University, Vocational School, Food Processing Department, İzmir, Türkiye
[3]Ege University, Engineering Faculty, Food Engineering Department, İzmir, Türkiye
91230000177@ogrenci.ege.edu.tr (Presenter and corresponding author, Zahide Dündar)

ABSTRACT

Palm oil is a vegetable oil used for many food and non-food applications. The global demand for palm oil will continue to rise, owing to the growing population and expanding economy. To meet increasing demand, the area of land dedicated to palm oil production in producer countries in Southeast Asia such as Malaysia and Indonesia has increased steadily. Palm oil establishment in these areas has resulted in widespread deforestation, deterioration of ecological balance, and increased greenhouse gas emissions. To avoid these problems and fulfill the growing global demand for palm oil, voluntary certification under the International Roundtable Palm Oil (RSPO) and Malaysian Sustainable Palm Oil (MSPO) emerges as an efficient solution. These certifications aim to ensure sustainability, optimization of productivity and efficiency while adhering to transparency, ethical, and legal principles, respecting communities, supporting smallholders, and protecting workers while conserving the ecosystem. A related study examines the impact of sustainability-focused certification on product quality characteristics and stakeholder awareness in palm oil production. An assessment was conducted with approximately 104 individuals involved in the palm oil supply chain, either as producers or users. The assessment covered recognition of sustainability concepts, awareness of health benefits associated with palm oil and brand perception about certification in the context of purchasing products containing palm oil. This study has the potential to serve as a model for studies that will contribute to the advancement of conscious consumption and circular production in palm oil sustainability certification parameters. Preliminary results revealed that producers and consumers are aware of sustainability. Moreover, sustainability certification might positively affect brand recognition and purchase tendency for palm oil products. Developing efficient marketing and informative advertising based on scientific facts is crucial to maintaining and expanding awareness of the sustainability concept of palm oil and its products.

INTRODUCTION

Palm oil, widely used in food, cosmetics, and biofuels, has become a critical subject in environmental and health discussions due to its association with deforestation, biodiversity loss, and greenhouse gas emissions (Wiebe et al., 2019). To mitigate these concerns, sustainability certifications such as the Roundtable on Sustainable Palm Oil (RSPO) have been developed, aiming to promote environmentally responsible palm oil production (RSPO, 2021). Upon the demand of consumers for the use of certified palm oil; the Indonesian government introduced the Indonesian Sustainable Palm Oil (ISPO) standards in 2011, and the Malaysian government introduced the Malaysian Sustainable Palm Oil (MSPO) standards in 2015. In this direction, the establishment of well-planned and traceable palm oil production has a great determination on the protection of the environmental ecosystem in safe and healthy production (Ivancic and Koh, 2016). As of December 31, 2019, MSPO has become mandatory for all Malaysian palm oil producers and 639,407.61 hectares were registered as independent smallholders' plantations, 444,872.36 hectares were registered as organized smallholders' plantations, and 3,600,781.46 hectares were registered as total certified plantations. (MPOCC, 2024a). Making MSPO mandatory ensures that producers adopt a standard; therefore, a rapid increase has been achieved. The main objectives of the MSPO certification include; management commitment and responsibility, health worker safety and employment conditions, compliance with legal requirements, development of new plantations, and transparency (MPOCC, 2024b).

However, the success of these initiatives largely depends on consumer awareness and understanding of the sustainability issues surrounding palm oil (Taufique and Vaithianathan, 2018). Research has shown that socio-demographic factors like education and income significantly influence consumer behavior towards sustainable products (Diamantopoulos et al., 2003; Zhao et al., 2014). Moreover, gender differences have also been observed, with women generally displaying higher environmental awareness than men (Hunter et al., 2004; Zelezny et al., 2000).
Consumers' understanding of sustainability certifications plays a vital role in shaping purchasing behavior. While some studies indicate that sustainability labels influence purchasing decisions, knowledge gaps regarding these certifications persist, limiting their effectiveness. (Grankvist et al., 2004; Hartmann and Apaolaza-Ibáñez, 2012). Younger and middle-aged adults, typically more engaged in sustainability issues, are key drivers of demand for eco-friendly products.
This study seeks to assess consumer awareness and perceptions of palm oil, focusing on the acceptability of palm oil, knowledge of its uses, and the role of sustainability certifications in shaping purchasing decisions. By surveying a diverse sample, the study provides insights into consumer behavior and the factors influencing sustainable purchasing trends.

MATERIAL AND METHODS

This study employed a cross-sectional survey design to collect data on consumer awareness, perceptions, and behaviors related to palm oil and sustainability certification awareness. The survey was distributed online and person to person, simultaneously to a diverse sample of 104 participants across various demographics. The questionnaire included sections on general awareness of palm oil (e.g., familiarity with palm oil used in products), understanding of sustainable palm oil (e.g., knowledge of RSPO certification), and purchasing behavior (e.g., the influence of sustainability claims on buying decisions). Participants were also asked to rate the quality of palm oil-containing products and to identify differences between palm oil and other related oils. All responses were examined and presented as percentages on graphs.

RESULTS AND DISCUSSION

The results of the survey are shown in graphs in Figures. According to the findings given in Figure 1, the survey's participant profile, predominantly female (68.26%) and concentrated in the 25-44 age range, reflects a well-educated and relatively affluent group, with 52.88% holding a master's degree or higher and 40.38% reporting high income. This demographic, often linked to greater environmental awareness and sustainable consumption behaviors, likely contributed to the high levels of awareness and preference for sustainable palm oil observed in the survey, indicating a group well-positioned to make informed, eco-conscious purchasing decisions.

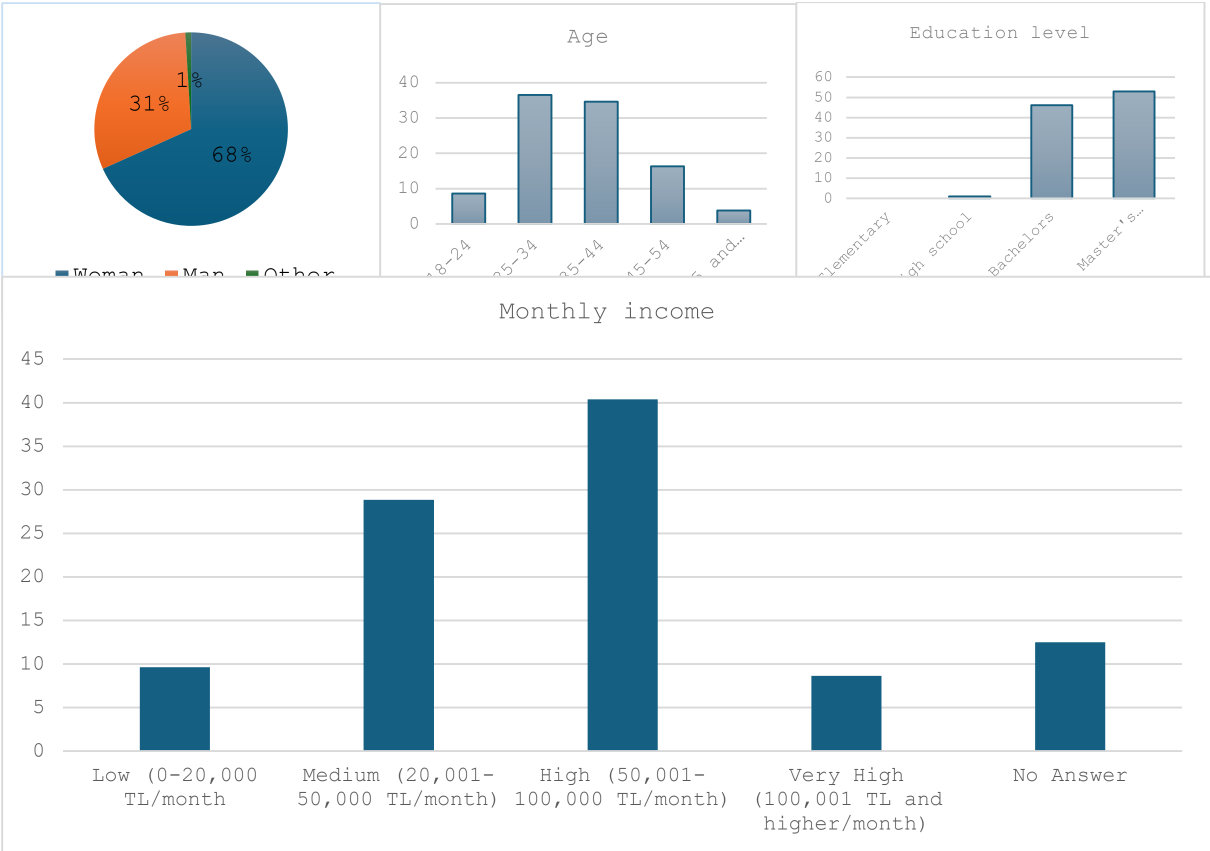


Figure 1. General information about the survey participants

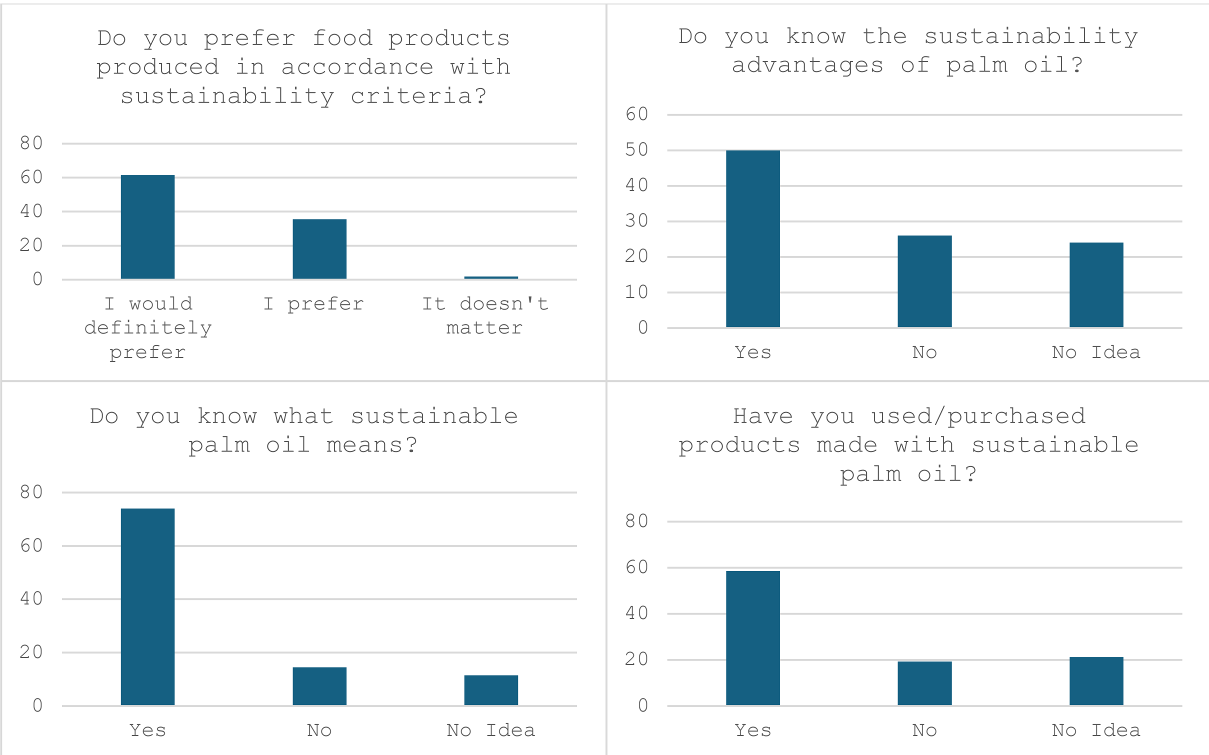
The demographic profile of the survey participants, including their higher education levels, income, and gender distribution, aligns with findings from prior studies that associate these factors with increased environmental awareness and sustainable consumption behaviors. For instance some previous studies highlight that higher education and income are strong predictors of environmental concern and the likelihood of purchasing sustainable products (Diamantopoulos et al., 2003; Zhao et al., 2014). Additionally, in some previous research, it was reported that women tend to show greater environmental awareness, which could explain the higher proportion of female respondents in the survey (Hunter et al., 2004; Zelezny et al., 2000). The predominance of young to middle-aged adults, who are typically more engaged in sustainability issues, further supports the survey's findings of heightened awareness and preference for sustainable palm oil among this demographic (Liere and Dunlap, 1980).

Figure 2. Palm oil acceptability of the customers

The results on palm oil acceptability among customers reveal a complex perception of this ingredient, which is influenced by various factors such as health concerns, awareness of its uses, and sustainability issues (Figure 2). While a significant portion of respondents (57.69%) do not believe palm oil is harmful to health, there is still a notable minority (30.76%) who perceive it as harmful, reflecting ongoing public debates and mixed information about palm oil’s health impacts. Awareness of palm oil's uses is high, with 86.53% of respondents familiar with its applications, indicating that most consumers are well-informed about the prevalence of palm oil in products. However, there is ambiguity regarding the differences between palm oil and other oils, such as "hurma" oil, with respondents almost evenly split on this question, suggesting some confusion or lack of detailed knowledge about different types of oils. This response actually conflicts with the previous findings about how consumers are aware of palm oil, and its uses since “hurma oil” and “palm oil” technically address the same oil. Accordingly, it has been observed that a significant portion of consumers think that they have detailed information about palm oil and its uses, but even this simple difference in expression misleads consumers. This suggests that some marketing-oriented initiatives, such as advertising campaigns, significantly impact consumer perceptions.

These perceptions are crucial because they directly affect consumer behavior. For instance, while many consumers know palm oil and its uses, their health concerns and understanding of its sustainability aspects play a significant role in their purchasing decisions. The survey also shows that environmental sustainability (35.29%) and healthy living (32.35%) are key factors influencing purchasing decisions for products containing palm oil, reflecting a growing consumer trend toward products that align with personal health and environmental values. This aligns with previous studies, highlighting health and sustainability's increasing importance in consumer decision-making processes (Hartmann and Apaolaza-Ibáñez, 2012). Overall, the results suggest that while many consumers generally accept palm oil, its acceptability is contingent upon ongoing concerns and knowledge gaps related to health and sustainability, which can significantly influence consumer preferences and behavior.

Although environmental issues positively affect the consumption of products containing palm oil, it has been seen that it is necessary to evaluate the price factor, which has a share in the consumption phenomenon, together with the product quality. When examined in this direction, the findings indicate a balance between the price and quality of palm oil products. The cost reduction in the prices of palm oil products has not been reflected in their quality at the same rate, and it has been determined that the effect of quality is moderate. The reason for this finding can be shown as the compatibility of palm oil processing methods with sustainability principles (Panapanaan et al., 2009; Gassler and Spiller, 2018).



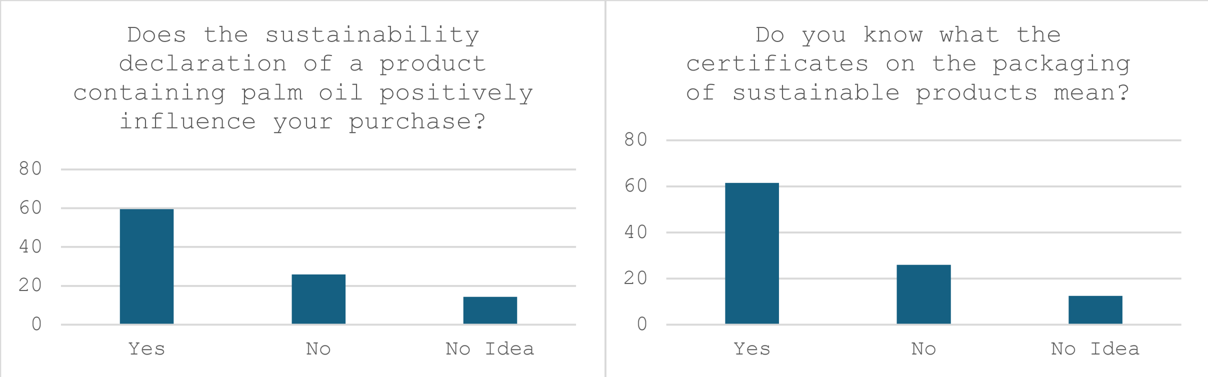


Figure 3. Sustainability awareness and sustainability certification on palm oil

The results on sustainability awareness and palm oil sustainability certification indicate a relatively high level of consumer engagement with sustainability issues, though there are notable gaps in understanding (Figure 3). A significant majority of respondents (74.03%) reported knowing what sustainable palm oil means, and 50% were aware of the specific sustainability advantages associated with palm oil. This level of awareness reflects a growing consumer consciousness around sustainability, particularly in relation to palm oil, which has been widely discussed in environmental and ethical contexts due to its impact on deforestation and biodiversity.

However, despite this awareness, there is still a considerable portion of the population that is either unaware or unsure about the specifics of sustainability certifications. For instance, 25.96% of respondents admitted they do not know what the certifications on product packaging imply, and 24.03% are unaware of the sustainability advantages of palm oil. This suggests that while consumers are increasingly aware of sustainability as a concept, their understanding of the detailed implications of certifications and specific sustainability benefits is incomplete.

The influence of sustainability certifications on purchasing behavior is also evident, with 61.53% of respondents indicating that such certifications positively influence their buying decisions. This aligns with findings from previous research, which demonstrates that sustainability labels and certifications can significantly affect consumer behavior, especially among those already inclined towards environmentally friendly practices (Taufique and Vaithianathan, 2018). Nonetheless, the knowledge gaps identified suggest that for certifications to be fully effective in driving consumer behavior, further efforts are needed to educate consumers on what these certifications represent and how they contribute to sustainable practices. Overall, the results highlight the critical role of informed consumer education in enhancing the impact of sustainability certifications on purchasing decisions.

CONCLUSIONS

The results highlighted the significant role of sustainability certifications in shaping consumer awareness and purchasing behaviors related to palm oil products. The findings indicate that while there is a high level of general awareness about sustainability, gaps remain in understanding specific certifications and their implications. Consumers, particularly those with higher education levels and incomes, prefer products aligned with environmental and health values. However, misconceptions and incomplete knowledge about palm oil and related products persist, suggesting more targeted educational efforts are needed. Future research should focus on exploring the long-term impact of sustainability education on consumer behavior and how marketing strategies can more effectively communicate the benefits of sustainable palm oil. Additionally, examining the role of pricing and quality perception in sustainable product choices could provide deeper insights into consumer decision-making processes.

ACKNOWLEDGMENTS

This study was conducted with the permission of Ege University Scientific Research and Publication Ethics Committees (EGEBAYEK).

REFERENCES

Diamantopoulos, A., Schlegelmilch, B. B., Sinkovics, R. R., Bohlen, G. M. 2003. "Can socio-demographics still play a role in profiling green consumers? A review of the evidence and an empirical investigation". Journal of Business Research, 56(6), 465–480.

Gassler, B., Spiller, A. 2018. "Is it all in the MIX? Consumer preferences for segregated and mass balance certified sustainable palm oil". Journal of Cleaner Production, 195, 21-31.

Grankvist, G., Dahlstrand, U., Biel, A. 2004. "Gunne Grankvist , Ulf Dahlstrand and Anders Biel The Impact of Environmental Labelling on Consumer Preference : Negative vs . Positive Labels". Journal of Consumer Policy, 27, 213–230.

Hartmann, P., Apaolaza-Ibáñez, V. 2012. "Consumer attitude and purchase intention toward green energy brands: The roles of psychological benefits and environmental concern". Journal of Business Research, 65(9), 1254–1263.

Hunter, L. M., Hatch, A., Johnson, A. 2004. "Cross-national gender variation in environmental behaviors". Social Science Quarterly, 85(3), 677–694.

Ivancic, H., & Koh, L. P. (2016). Evolution of sustainable palm oil policy in Southeast Asia. Cogent Environmental Science, 2(1), 1195032.

Liere, K. D. V., Dunlap, R. E. 1980. "The social bases of environmental concern: A review of hypotheses, explanations and empirical evidence". Public Opinion Quarterly, 44(2), 181–197.

MPOCC, 2024a, MSPO Trace, (Accession date: 28.08.2024), https://mspotrace.org.my/

MPOCC, 2024b, About MSPO, (Accession date: 28.08.2024), https://www.mpocc.org.my/about-mspo

Panapanaan, V., Helin, T., Kujanpää, M., Soukka, R., Heinimö, J., & Linnanen, L. 2009. "Sustainability of palm oil production and opportunities for Finnish technology and know-how transfer." Research Report/Lappeenranta University of Technology, Faculty of Technology, LUT Energy.

RSPO. 2021. "Roundtable on Sustainable Palm Oil: What we do.". Tarihinde adresinden erişildi https://rspo.org/who-we-are/

Taufique, K. M. R., Vaithianathan, S. 2018. "A fresh look at understanding Green consumer behavior among young urban Indian consumers through the lens of Theory of Planned Behavior". Journal of Cleaner Production, 183, 46–55.

Wiebe, K. D., Sulser, T. B., Pacheco, P., De Pinto, A., Mason-D'Croz, D., Dermawan, A., … Dunston, S. 2019. "The palm oil dilemma: Policy tensions among higher productivity, rising demand, and deforestation". IFPRI Policy Brief, (June).

Zelezny, L. C., Chua, P. P., Aldrich, C. 2000. "Elaborating on gender differences in environmentalism". Journal of Social Issues, 56(3), 443–457.

Zhao, H. H., Gao, Q., Wu, Y. P., Wang, Y., Zhu, X. D. 2014. "What affects green consumer behavior in China? A case study from Qingdao". Journal of Cleaner Production, 63, 143–151.

EFFECT OF STORAGE IN REFRIGERATOR SHELVES AND CARDBOARD VIOL ON EGG QUALITY AND SHELF LIFE

Fatma Yenilmez

Cukurova University, Vocational School of Tufanbeyli, Plant and Animal Production Department, Adana, Turkey.

Corresponding author e-mail: fyenilmez@cu.edu.tr

ABSTRACT

The egg is a food, that is extremely important for balanced nutrition and health for humans due to the nutrients it contains and its high biological value. And it is a protein source that can be easily consumed by people from all walks of life out of its affordable prices. Shelled eggs are one of the best-protected foods against microorganisms on account of their structure. However, the high nutritional value creates a suitable environment for the development of microorganisms and prepares the conditions for the egg to spoil. Therefore, to prevent spoilage, some precautions must be taken. Storing in cold conditions, especially at home in the refrigerator, is a common practice for this purpose. Egg storage conditions and duration are among the most important criteria affecting egg quality and shelf life. The current study investigated the effects of storing eggs in refrigerator shelves or cardboard viols on the internal and external quality criteria of eggs. For this purpose, a total of 125 eggs obtained from 44-week-old Lohman Brown hens were used. Within the scope of the study, the initial, 15th and 30th day internal and external quality characteristics of the eggs were determined. According to the research results, the effect of storing eggs in the refrigerator shelves and the cardboard viol on weight loss (g, %), albumen width, albumen length, albumen height, albumen weight (g), albumen index, pH, Haugh Unit was found to be statistically significant, while the effect on breaking resistance, shell weight (g, %), yolk weight (g, %), albumen weight (%), yolk height, yolk width, yolk index and yolk color *L*, *a** and *b** values was found to be insignificant. Egg weight loss, yolk and albumen index and Haugh Unit were found to be higher in eggs stored in the refrigerator shelves, while pH was higher in eggs stored in the cardboard viols. Although weight loss was higher in eggs stored in the refrigerator shelves, they were of better quality and darker color than eggs stored in the cardboard viols. In light of these results, it is recommended to store eggs on refrigerator shelves without using cardboard viols in terms of the shelf life of eggs.

Keywords: Internal-external quality, egg, storage in the refrigerator, shelf life

INTRODUCTION

Eggs are a precious food in terms of the diversity and biological value of the nutrients in their structure. Especially, the fact that people of all ages can easily consume it and that it is easily accessible puts it at the forefront among other foods. The protection and storage conditions of this special food are very important in terms of preventing it from deteriorating.

Shelled eggs are one of the foods that are best protected against microorganisms due to their natural structure. Fresh eggs are generally considered sterile when they are first laid. In later times, the eggshell may become contaminated with microorganisms due to feces, soil and infections in the chicken. While the egg is forming, it is covered with a layer called cuticle. The cuticle consists of 85-90% protein, some carbohydrates and a small amount of lipids. The cuticle dries immediately after the egg is laid and prevents the entry of bacteria and the exit of water from the egg thanks to its selectively permeable structure (Baker and Balc, 1962). The calcified shell and shell membranes that form the shell of the egg together with the cuticle form a natural physical and chemical barrier against contamination by microorganisms (Özçevik et al., 2023; Anonymous, 2024).

Another protective system is the lysozyme enzyme found in the albumen, which is particularly effective against gram-positive bacteria. In addition, avidin combines with biotin in the albumen to prevent microorganisms from using biotin. Similarly, conalbumin forms a complex with iron, preventing microorganisms from using iron (Anonymous, 2024).

Eggs are a perishable product. Despite being naturally so protected, if they are not stored correctly, their quality decreases. Shortly after laying, changes begin to be observed inside the egg, depending on the ambient temperature and storage time. CO2 and weight loss occur, albumin height and Haugh Unit decrease, yolk weight and pH value increase (Yılmaz and Bozkurt 2009; Brandao et al., 2014; Şekeroğlu et al., 2016; Saleh et al., 2020; Yenilmez et al., 2024).

Different methods are used to preserve the freshness of eggs and prevent the proliferation of microorganisms. It has been observed that these methods have positive effects on preserving egg quality. Cold storage is the most common of these and helps preserve egg quality, prevents microorganism growth and reduces CO_2 loss (Rocha et al., 2013). Although preservation of egg quality depends on the storage period, storage conditions at 10–13 °C and 80–85% humidity are considered sufficient (Keener et al., 2006; Altan 2015). Other methods of preserving eggs are coating with various materials such as propolis (Copur et al. 2008), glycerin (Drabik et al., 2018), different oils (Nongtaodum et al., 2013), lipids, polysaccharides, proteins and plastic, and storing in different atmospheric conditions (Altan 2015; Da Silva Pires et al., 2020; Rachtanapun et al., 2021; Şahansoy et al., 2024).

It is observed that there are many different packaging materials used in the packaging of eggs. Styrofoam, plastic and fiber cardboards, plastic and fiber trays, and plastic packaging bags are used for this purpose. Molded pulp cardboard or polystyrene foam cardboards are used all over the world due to their low cost. The advantage of using polystyrene material is that it has superior cushioning properties, protection against odor and moisture, and is resistant to fungus and mold formation. Another packaging material is transparent plastic packaging that shows the inside. Since the eggs are visible, consumers do not need to open them to inspect (Yüceer and Caner, 2021).

The most commonly used material for egg packaging in Turkey is wrapping eggs in cardboard vials with transparent stretch film (Yılmaz and Bozkurt 2009). Eggs brought home are removed from the stretch film and stored either in cardboard vials or on refrigerator shelves. In the current study, the effects of storing eggs in refrigerator conditions in vials or open on a cabinet shelf on egg quality were investigated.

MATERIAL AND METHOD

A total of 125 eggs obtained from 44-week-old Lohman Brown hens were used in the study. The eggs were provided by the Cukurova University Poultry Department. After the weights of the eggs were taken with a precision scale, 25 of them were separated to determine the initial internal-external quality criteria, and the remaining eggs were randomly distributed equally into 2 groups (50+50). One of the groups was stored in the refrigerator (+4°C) for 30 days in the cardboard vials in which eggs are commonly packaged and sold, and the other group was stored in the open on the shelf section of the refrigerators where eggs are arranged for 30 days (+4°C).

Internal and external quality analyses were performed on days 0., 15. and 30. Egg weight, shell weight, shell percentage, and shell breaking resistance (Texture Analyzer; Stable Micro Systems_TA.XT Plus, England) were measured as external quality characteristics in the eggs. Internal quality characteristics yolk height, yolk width, albumen height, albumen width, albumen length, albumen pH, yolk color *L, a** (redness) and *b** (yellowness) values (HunterLab, ColorFlex EZ, United States), albumen and yolk weight were determined. Albumen weight and percentage, yolk percentage, shell percentage, albumen index, yolk index and Haugh Unit were calculated using the following formulas.

Albumen Weight: *[Egg weight-(Yolk weight+Shell weight)]*

Albumen Ratio (%): *[100-(Yolk ratio+Shell ratio)*

Yolk Ratio (%): *[Yolk weight/Egg weight] x 100*

Shell Ratio (%): *[Shell weight/Egg weight] x100*

Yolk Index: *[Yolk height/Yolk diameter]x100*

Albumen Index: *[Albumen height/{(Albumen length+ Albumen width)/2}]x100*

Shell Thickness: *[Blunt part+Pointed part+Equatorial part]/3*

Haugh Unit: *100 log [Albumen height+7.57–1.7xEgg weight* $^{0.37}$*]*

The data obtained in the study were analyzed using the IBM SPSS 19.0 (2010) package program. Normal distribution of the data was made using variance analysis (ANOVA) and the Tukey test was used to compare the means. The statistical significance level was accepted as P ≤0.05 and the means are presented in the table as mean±SE (Standard Error).

RESULTS AND DISCUSSION

According to the research results, the effect of storing eggs on the refrigerator shelves and in the cardboard vial on weight loss (g, %), albumen height, albumen length, albumen index, pH, Haugh unit (P<0.01) and albumen width and albumen weight (g) (P<0.05) values was found to be statistically significant, while the effect on breaking strength, shell weight (g, %), yolk weight (g, %), albumen weight (%), yolk height, yolk width, yolk index and yolk color *L*, *a** and *b** was found to be insignificant (Table 1).

Tablo 1. Effect of storage in the refrigerator shelves and cardboard viol on egg quality (mean±SE)

Parameters	0. Day	Refrigerator Shelves		Cardboard Viols		P
		15. Day	30. Day	15. Day	30. Day	
Egg weight loss (g)	-	0.67±0.02	1.31±0.17	0.55±0.02	1.14±0.05	0<01
Egg weight loss %	-	1.09±0.03	2.20±0.29	0.94±0.03	1.92±0.07	0<01
Breaking strength (kg/cm²)	3.69±0.19	3.67±0.20	3.50±0.18	3.99±0.19	3.48±0.39	0.444
Eggshell weight (g)	5.87±0.12	5.92±0.11	5.96±0.08	5.97±0.10	5.78±0.15	0.838
Eggshell weight (%)	9.66±0.22	9.77±0.20	10.21±0.17	10.26±0.14	9.98±034	0.126
Yolk weight (g)	15.91±0.18	16.47±0.30	16.47±0.22	16.40±0.92	16.04±0.29	0.199
Yolk weight (%)	26.17±0.36	27.27±0.54	26.83±1.06	27.10±0.84	27.71±0.73	0.678
Albumen weight (g)	39.17±0.69	38.17±0.72	36.86±0.82	36.52±0.88	36.20±1.27	0.053
Albumen weight (%)	64.17±0.45	62.96±0.66	62.95±1.09	62.65±1.19	62.30±0.86	0.457
Yolk height (mm)	18.28±0.13	18.67±0.18	17.88±0.13	18.46±0.20	17.49±0.32	0.887
Yolk width (mm)	40.68±0.34	38.99±0.32	41.25±0.26	39.13±0.36	40.77±0.34	0.055
Albume height (mm)	8.29±0.18	6.61±0.25	5.73±0.17	6.06±0.18	5.47±0.24	0<01
Albume width (mm)	68.99±0.77	74.12±1.49	74.41±1.20	70.65±3.01	72.42±1.56	0.028
Albume length (mm)	81.95±1.05	89.64±1.55	95.11±1.66	87.62±1.61	96.00±2.31	0<01
Yolk index	44.99±0.44	48.06±0.57	43.42±0.42	45.55±2.00	42.94±0.88	0.950
Albumen index	11.04±0.31	8.24±0.43	6.90±0.27	7.86±0.39	6.55±0.40	0<01
pH	7.69±0.04	8.79±0.02	8.47±0.08	8.83±0.02	8.52±0.03	0<01
Haugh Unit	90.72±0.91	80.05±1.67	74.36±1.38	77.23±1.30	72.90±1.88	0 <01
L	54.77±0.58	53.68±0.51	54.00±0.40	53.35±0.56	55.65±0.55	0.355
a*	18.91±0.56	18.59±0.37	19.18±0.34	19.08±0.43	19.30±0.70	0.902
b*	53.32±0.21	51.79±0.69	56.79±0.47	55.16±1.03	56.82±1.26	0.172

L: Brightness, *a*:* Redness, *b*:* Yellowness

Egg quality is affected by some factors such as genetics, age of the hen, stages of the laying cycle, nutritional level, conditions in the coop, management system, housing system selected, and diseases before laying (Abebe et al., 2023). After laying, storage practices have a significant effect on egg quality (Gerber, 2010) and even short-term storage affects quality (Gavril and Usturoi (2011). Janes et al. (2018) reported that storing eggs at 4°C did not affect egg quality factors. While Kim et al. (2024) reported that storing eggs under refrigerator conditions improves egg quality, Usturoi et al. (2014) stated that table eggs should be stored under refrigerator conditions.

The internal quality of eggs starts to decrease immediately after laying (Roxana and Usturoi, 2012). The longer the storage period, the worse the internal quality of the egg. This is because the loss of carbon dioxide from the eggshell is facilitated by temperature and humidity (Oliveira; Oliveira, 2013). Raji et al. (2009) reported that egg quality decreases with storage period and egg weight decreases during storage, Siyar et al. (2007) reported that egg weight decreases significantly with storage period, and Tayeb (2012) stated that there is an increasing weight loss with increasing storage period. In this study, weight loss decreased in proportion to the time in eggs stored both on the refrigerator shelves (0.67±0.02, 1.31±0.17 g) and in cardboard viols (0.55±0.02, 1.14±0.05 g). The quality and freshness of table eggs are determined by the Haugh unit value, which is determined using egg weight and albumen height (Skřivan et al., 2006). Eggs stored in cardboard viols lost less weight in grams and percentages than those stored on refrigerator shelves and remained fresher (Figure 1).

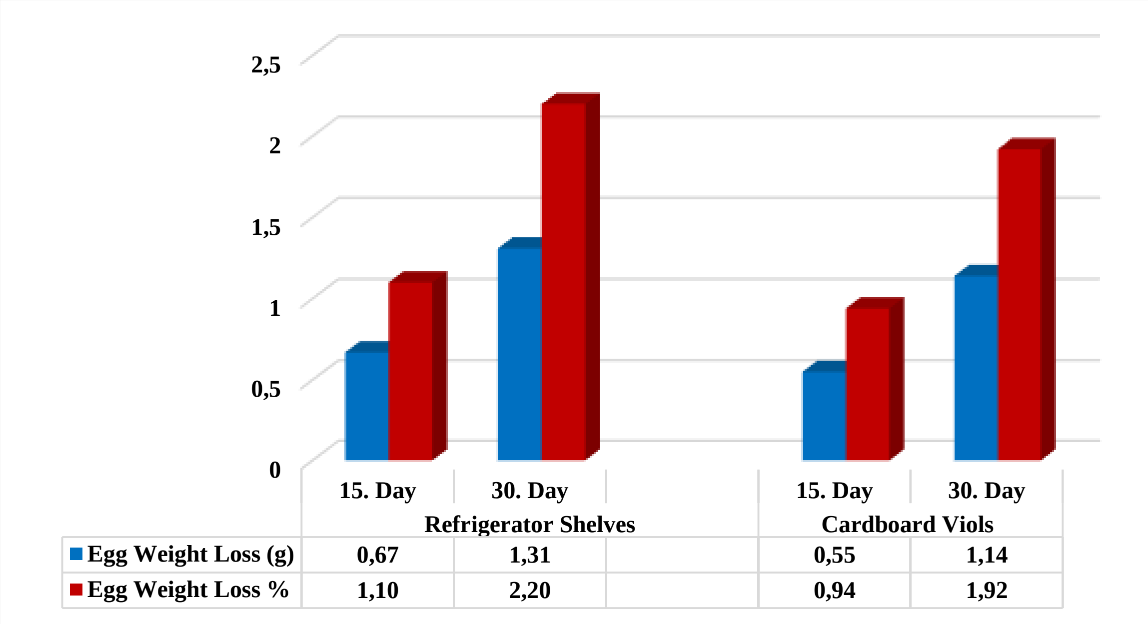


Figure 1. Weight loss in eggs stored in the refrigerator shelves and cardboard viols

Yamak et al. (2021) grouped organic and cage-produced eggs as stretched, unstretched and washed, and stored them in room and refrigerator conditions. The lowest weight loss was in eggs stretched and stored in the refrigerator, and the highest was in washed and stored in the room. In our study, weight loss was found to be less in eggs stored in cardboard viols.

The average albumen height in fresh eggs is 7.54 mm. Siyar et al. (2007) reported that albumen height was significantly affected by storage time, while Raji et al. (2009) stated that it decreased with storage time. Yamak et al. (2021) found the highest albumen height in eggs stored in the refrigerator. In the present study, albumen height was found to be higher in eggs stored on the refrigerator shelves on the 0th, 15th and 30th days (8.29±0.18, 6.61±0.25 and 5.73±0.17) than in eggs stored in cardboard viols (8.29±0.18, 6.06±0.18 and 5.47±0.24).

Siyar et al. (2007) and Khan et al. (2013) stated that albumen weight decreased significantly with storage time. In the present study, albumen weight in eggs stored in cardboard viols was 39.17±0.69, 38.17±0.72 and 36.86±0.82 g on days 0, 15 and 30, respectively, while it decreased over time in eggs stored in refrigerator shelves to 39.17±0.69, 36.52±0.88 and 36.20±1.27 g respectively.

Raji et al. (2009) found that albumen length increased during storage. Our research results were consistent with the literature, albumen length increased with storage time in both groups (refrigerator shelves: 81.95±1.05, 89.64±1.55, 95.11±1.66, cardboard viols: 81.95±1.05, 87.62±1.61, 96.00±2.31). The egg yolk is round and located in the middle of fresh eggs. Over time, as the vitelline membrane degenerates, the egg yolk absorbs water from the egg white, loses its spherical appearance, takes a flattened shape and decreases in height (Anonymous, 2024; Tayar, 2015). Yolk height decreased in both the eggs stored on the refrigerator shelves on the 0th, 15th and 30th days (18.28±0.13, 18.67±0.18, 17.88±0.13) and in the eggs stored in the cardboard vial (18.28±0.13, 18.46±0.20, 17.49±0.32), as reported by Raji et al. (2009).

Khan et al. (2013) reported that different storage periods caused a decrease in the albumen index and yolk index of Fayoumi eggs, Shehata et al. (2023) stated that the yolk index decreased over time, and Raji et al. (2009) also reported decreases in albumen and yolk index during storage. The yolk index was higher in eggs stored on the refrigerator shelves than in the cardboard vial (44.99±0.44, 48.06±0.57, 43.42±0.42) on days 0, 15 and 30 (Figure 1). Similarly, the albumen index was higher in eggs stored on the refrigerator shelves (11.04±0.31, 8.24±0.43, 6.90±0.27) than in the cardboard vials (11.04±0.31, 7.86±0.39, 6.55±0.40).

The rheological properties of albumin, emulsification ability and whipping and gelling properties of eggs are important criteria in the egg products industry (Kemp et al., 2010). The important difference between fresh and stored eggs is albumin pH and egg albumin quality (Nadia et al., 2012). With the increase in storage time, mucin, which gives albumin a mushy structure, loses its structural feature and the albumin becomes watery and albumen height decreases (Şenköylü, 1991). Albumen height, albumen index, Hough unit and pH values, which are the most important indicators of internal quality characteristics, gave better results in eggs stored on the refrigerator shelves than in cardboard viols.

Albumen pH value was higher in eggs stored in cardboard viols (7.69±0.04, 8.83±0.02, 8.52±0.03), while it was lower in eggs stored on the refrigerator shelves (7.69±0.04, 8.79±0.02, 8.47±0.08) (Table 1, Figure 2). Albumen pH in fresh eggs was reported to be between 7.6-8.5. As eggs age, the dense albumen becomes liquid due to numerous chemical reactions occurring in it, probably including the formation of carbonic acid (H_2CO_3) and increasing albumen pH. H_2CO_3, one of the components of the albumin buffer system, dissociates to form water and carbon dioxide (CO_2). Under natural conditions, CO_2 and water present in the shell diffuse and evaporate through the shell pores, decreasing albumen acidity, increasing pH and chemical disintegration of the protein complex. The loss of thickness of the dense albumen is related to the natural dissociation of this complex (Şenköylü, 1991; Oliveira and Oliveira, 2013). The effect of pH on egg quality is closely related to its freshness. It has been confirmed that albumen and yolk pH increases depending on storage time and storage temperature (Siyar et al., 2007; Garcia et al., 2010; Shehata et al., 2023). In the present study, the pH value was lower in the eggs stored on the refrigerator shelves and the eggs remained fresher (Figure 1).

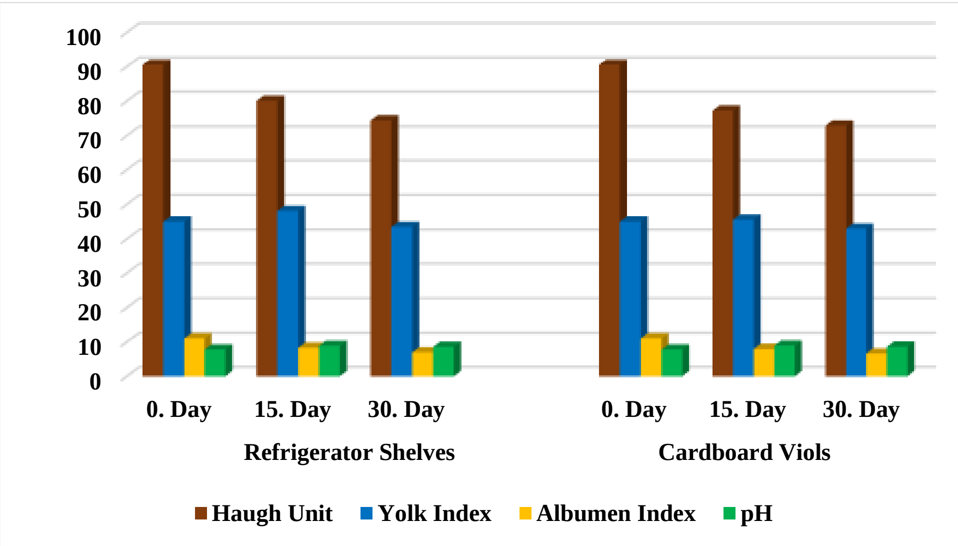


Figure 2. Changes in Haugh Unit, Yolk Index, Albumen Index and pH unit values in eggs stored in the refrigerator shelves and in the cardboard vial

Haugh unit is significantly affected by storage time (Siyar et al., 2007; Okonkwo et al., 2021) and gradually decreases with storage time (Raji et al., 2009; Alsobayel and Albadry, 2011; Tayeb, 2012; Khan et al., 2013; Shehata et al., 2023). Haugh unit was 90.72±0.91, 80.05±1.67, 74.36±1.38 in eggs stored on the refrigerator shelves at days 0, 15 and 30 and was 90.53±0.90, 77.23±1.30, 72.90±1.88 in eggs stored in cardboard viols. The quality of albumen is measured by the Haugh unit suggested by Raymond Haough. In table eggs, this value should be 79 or higher for AA quality eggs, 55-78 for A quality, 31-54 for B quality and 30 or less for C quality (Şenköylü, 1991). In the current study, the quality of eggs stored on the refrigerator shelves was AA quality on the 15th day but dropped to A quality on the 30th day. In eggs stored in cardboard viols, they maintained their A quality feature on the 15th and 30th days.

Egg yolk color is one of the most important parameters of egg quality about market requirements. In developing countries, consumers prefer egg yolks that are yellow-orange (Leeson and Summers, 2008). At the end of the study, the color of eggs stored in cardboard viols was darker (55.65±0.55), while redness (19.30±0.70) and yellowness (56.82±1.26) increased with storage time (Figure 3).

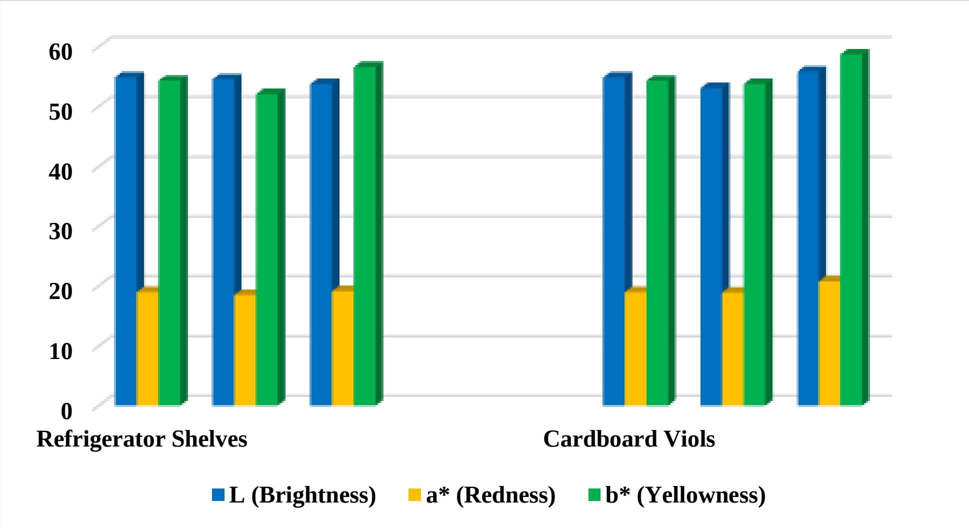


Figure 3. Changes in *L, a** and *b** values of yolk color in eggs stored in the refrigerator shelves and in the cardboard vial

Siyar et al. (2007) reported in their study that egg yolk and shell weight were not affected by storage time, while Khan et al. (2013) found that different storage times caused a decrease in the egg yolk weight of Fayoumi eggs. In the present study, yolk and shell weight were not affected by storage time in both groups.

CONCLUSIONS

The pH of eggs held in cardboard viols was greater, while the egg weight loss, yolk and albumen index, and Haugh Unit were found to be higher in eggs kept on refrigerator shelves. On day 15, the eggs kept in the refrigerator were of AA quality; however, by day 30, they had degraded to A quality. On days 15 and 30, the eggs in cardboard viols remained in their A quality attribute. Keeping table eggs on refrigerator shelves without using cardboard viols is healthier in terms of egg quality.

REFERENCES

Abebe, F., Mulatu, H., Kelemework, S. 2023. Review of Factors Affecting Egg Quality and Its Effect. Journal of Animal Health. 3(2): 17-32.

Altan, Ö. 2015. Yumurta: Oluşumu Kalitesi ve Biyoaktif Komponentleri. Ege Üniversitesi Basımevi, İzmir, Türkiye. 400 Sayfa.

Anonymous, 2024. Yumurtadaki Doğal Antimikrobiyal Sistemler. Available online: https://mobil.diatek.com.tr/Makale-Yontem/Mikrobiyolojik-Analiz/Yumurtada-Gorulen-Bozulmalar_3425.htm (accessed on: 11 July 2024).

Baker J. R., D. A. Balch. 1962. A Study of the Organic Material of Hen's-Egg Shell. Biochemistry Journal. 82, 352-361. https://doi.org/10.1042/bj0820352

Brake, J., T. J., Walsh, C. E., Benton, J. N., Petitte, R., Meijerhof , G., Penalva. 1997. Egg Handling and Storage. Poultry Science. 76: 144-151.

Brandao, M. D., F. F., Santos, L. S., Machado, M. S., Verinaud, J. M. Oliveira, N. M. Soares, E. R. Nascimento, V. L. A. Pereira. 2014. The Effect of Eggshell Apex Abnormalities on Table Egg Quality During Storage in 2 Seasons of the Year. Poultry Science. 93:2 657-2662. https://doi.org/10.3382/ps.2014-03991

Gerber, N. 2012 Factors affecting egg quality in the commercial laying hen. A review. Available from: http://eggfarmers.org.nz/eggfarmers/wpcontent/uploads/2012/04/ factors affecting egg quality.pdf. (Accessed: 24.08.2024).

Garcia, E. R. de M., C. C. O. Orlandi, C. A. L. de Oliveira, F. K. da Cruz, T. M. Baptista dos Santos, L. K. Otutumi. 2010. Quality of eggs of lay hens stored at different temperature and storage conditions. Revista Brasileira de Saúde e Produção Animal, 11(2), 505-518.

Jones, D. R., G. E. Ward, P. Regmi, D. M. Karcher. 2018. Impact of egg handling and conditions during extended storage on egg quality. Poultry Science. 97(2), 716-723. https://doi.org/10.3382/ps/pex351.

Keener, K., K. McAvoy, J., Foegeding, P., Curtis, K., Anderson, L. A. Osborne. 2006. Effect of Testing Temperature on Internal Egg Quality Measurements. Poultry Science. 85:550-555. https://doi.org/10.1093/ps/85.3.550

Kemp, B. J., F. R. Bamelis, K. Mertens, E. M. Decaypere, J. G. D. Baerdemaeker, B. D. Ketelaer. 2010. The assessment of viscosity measurements on the albumen of consumption eggs as an indicator for freshness. Poultry Science. 89(12): 2699-2703.

Kim, Y. B., S. Y. Lee, K. H. Yum, W. T. Lee, S. H. Park, Y. H. Lim, N. Y. Choi, S. Y. Jang, J. S. Choi,, J. H. Kim. 2024. Effects of storage temperature and egg washing on egg quality and physicochemical properties. Discover Applied Sciences. 6(3). https://doi.org/10.1007/s42452-024-05760-1.

Leeson, S., J. D. Summers. 2008. Commercial poultry nutrition 3rd edn Nottingham University Press, Thrumpton, Nottingham.

Nadia, N. A. A., S. R. Z., Bushra, A. F., Layla, M. A., Fira. 2012. Effect of Coating Materials (gelatin) and Storage Time on Internal Quality of Chicken and Quail Eggs under Refrigeration Storage. Poultry Science. 32 (1): 107-115.

Okonkwo, J. C., O. Chiamaka, I. F. Okonkwo, B. N. Marire. 2021. Effect of Storage Method and Storage Duration on Chicken Egg Quality. International Journal of Environmental & Agriculture Resear. 7(8): 13-16.

Oliveira, B. L. de, D. D. de Oliveira. 2013. Qualidade e Tecnologia de Ovos. 1. ed. Lavras, MG: UFLA, 223p.

Özçevik, İ., S., Korkmaz, O., Korkmaz. 2023. The Structure and Microbial Contamination Routes of Eggshell. Journal of Animal Production, 64 (1): 59-65 https://doi.org/10.29185/hayuretim.1003724

Pires, P. G., P., Pires, K., Cardinal, C., Bavaresco. 2020. The Use of Coatings in Eggs: A Systematic Review. Trends in Food Science & Technology. 106: 312-321. https://doi.org/10.1016/j.tifs.2020.10.019.

Rachtanapun, P., N., Homsaard, A., Kodsangma, N., Leksawasdi, Y., Phimolsiripol, S., Phongthai, J., Khemacheewakul, P., Seesuriyachan, T., Chaiyaso, S., Chotinan, P., Jantrawut, W., Ruksiriwanich, S., Wangtueai, S. R., Sommano, W., Tongdeesoontorn, K., Jantanasakulwong. 2021. Effect of Egg-Coating Material Properties by Blending Cassava Starch with Methyl Celluloses and Waxes on Egg Quality. Polymers, 13(21): 3787. https://doi.org/10.3390/polym13213787

Rocha, J. S. R., N. C., Baiao, V. M., Barbosa, M. A., Pompeu, M. N. S., Fernandes, L. J. C. Lara, C. F. Q., Matias, J. V. M. S. P. Batista. 2013. Negative Effects of Fertile Egg Storage on the Egg and the Embryo and Suggested Hatchery Management to Minimize Such Problems. World Poultry Science Journal. 69: 35-44.

Roxana, G., M. G., Usturoi. 2012. Effect of Storage Time and Temperature on Hen Egg Quality. Available online: http//www./univagrorasi-ro/revista200/20/document/ pdf-vol57/Roxana Garvid (accessed on: 15 July 2024).

Sharaf Eddin, A., S. A., Ibrahim, R., Tahergorabi. 2019. Egg Quality and Safety with an Overview of Edible Coating Application for Egg Preservation. Food Chemistry. 296: 29-39.

Shehata, E. R., Kenawi, M. A., El-Sokary, F. A. H., Abdel-Hamied, S. M. 2023. Assiut Journal of Agricultural Sciences 54 (1): 161-173. https ://doi.org/10.21608/ajas.2023.182200.121

Siyar, H. S. A., H., Aliarabi, A., Ahmadi, N., Ashori. 2007. Effect of Different Storage Conditions and Hen Age on Egg Quality Parameters. Australian Poultry Science Symposium, pp.106-109.

Skřivan, M., J., Simane, G., Dlouhá, J., Doucha. 2006. Effect of Dietary Sodium Selenite, Se-enriched Yeast and Se-enriched Chlorella on Egg Se Concentration, Physical Parameters on Eggs and Laying Hens Production. Czech Journal of Animal Science. 51: 163-167.

Şahansoy, H., C., Caner, M., Yüceer. 2024. The Shellac and Shellac Nanocomposite Coatings on Enhanced the Storage Stability of Fresh Eggs for Sustainable Packaging. International Journal of Biological Macromolecules. 261(1): 129817. https://doi.org/10.1016/j.ijbiomac.2024.129817.

Şekeroğlu, A., H., Gök, M., Duman. 2016 Effect of Egg Shell Color and Storage Duration on External and Internal Egg Quality Traits of ATAK-S Layer Hybrids. Ciencia e Investigacion Agraria. 43: 327-335. https ://doi.org/10.4067/S0718-16202016000200015

Şenköylü, N. 1991. Modern Tavuk Üretimi. Çiftlik Yayıncılık, Onaran Matbaa, Tekirdağ, Tütkey. 463 sayfa.

Yamak, U. S., M., Sarica, K., Ersoy, V., Ayhan. 2021. The Effects of Storage Conditions on Quality Changes of Table Eggs. Journal of Consumer Protection and Food Safety. 16: 71-81. https://doi.org/10.1007/s00003-020-01299-6

Yenilmez, F., A., Bulancak, N., Yılmaz, E., Uruk. 2024. Comparison of Quality and Microbiology of Four Genotype Eggs under the Different Storage Conditions. Çukurova Journal of Agriculture and Food Sciences. 39(1): 1-12.

Yilmaz, A., Z., Bozkurt. 2009. Effects of Hen Age, Storage Period and Stretch Film Packaging on Internal and External Quality Traits of Table Eggs. Animal Science and Biotechnology. 42: 462-469.

Yüceer, M., C., Caner. 2021. Bir ambalaj olarak yumurta viyolünün önemi ve sektördeki güncel gelişmeler. 26(270): 62-67. https://doi.org/10.13140/RG.2.2.21810.12489

THE ROLE OF ORGANIC FERTILIZER ON CROP PRODUCTION IN AFGOI-SOMALIA

Hawa Yahya AMİR[1], Hüseyin OK[1], İsmail Emrah TAVALI[2]

[1] Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Cyprus International University, 99258 Nicosia, Türkiye
[2] Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Akdeniz University, 07059 Antalya, Türkiye

* Correspondence: hok@ciu.edu.tr

ABSTRACT

The study used a descriptive approach and had 400 participants from Afgoi District Lower-Shebelle, Somalia. A non-simple random selection was used to acquire information from farmers, educators, and workers. To clarify the effect of naturally occurring fertilizer on crop productivity, the data were evaluated using correlation analysis. The majority of responders (56%) indicated significant agreement with the study's findings that green manure improves crop productivity by directly adding nitrogen to the soil, increasing soil fertility. The majority of responders (47.5%), however, were in agreement that plants grown in green manure receive nutrients from deeper levels of soil and release them in the topsoil. On the other hand, the majority of respondents (54%) firmly concur that green manure improves nutrient availability, both macro and micro. The majority of respondents (51%) firmly concur that green manures are environmentally benign under other circumstances. In addition, the majority of responders (57.5%) highly concur that green manure raises the caliber of products and crops. The majority of respondents (51.5%) indicated high agreement with the finding that adding manure to cropland can help keep or raise the level of soil organic matter. However, the majority of respondents (38%) agreed that animal manure includes both macro and micronutrients in both organic and inorganic forms that are essential for crop production. On the other hand, the majority of responders (45.5%) strongly concur that animal dung from a locally accessible source is cheaper than inorganic fertilizer when needed. Similar to this, the majority of respondents (41%) firmly concur that poultry manure is the best animal manure. However, the majority of respondents (51%) strongly concur that adding animal manure to the soil improves its quality. The majority of respondents (55%) said firmly that crop farming is the primary means of subsistence for Afgoi communities. However, the majority of respondents (37.5%) agreed that improvements have been made to the production system over the past two years. On the other hand, the majority of respondents (49.5%) agreed that using organic fertilizer increases crop output per unit of surface area. Similar to this, the majority of respondents (52.5%) strongly concur that fertilizer quality affects crop production.

Keywords: Animal Manure, Crop Production, Green Manure, Organic Fertilizer

INTRODUCTION

Organic fertilizers are crucial for the growth of plants. Organic fertilizers are typically more economical and easily accessible from local sources than chemical fertilizers (Solomon et al. 2012). The foundation of fertile soil is organic matter (Aboudrare 2009). Organic fertilizers are made from biological or living substances. These fertilizers provide nutrients to the soil gradually over time. Whereas artificially created fertilizers, or those obtained via the mining of non-living materials, are known as inorganic fertilizers. Inorganic fertilizers also referred to as chemical fertilizers, are utilized by plants very quickly. There are many different types of organic fertilizers, including Livestock manure from animals, including cows, poultry, goats, and others. Green manure is made from young plants, especially different kinds of legumes. Compost is made from organic agricultural waste like sorghum, maize stalks, or decaying garbage. Organic fertilizers such as sheep manure from farms and chicken manure may aid in crop growth as an alternative to artificial fertilizers (Khan et al. 2005). Organic fertilizer can improve soil fertility and aid in plant growth. Positive actions, including ammonium (NH_4^+), potassium (K^+), and magnesium (Mg_2^+), are held in biochar, which keeps these nutrients in the soil for an extended period of time and releases them to plants as needed (Lal 1982). The manure from the farm had a long-lasting influence on maize when administered to wheat-based in a wheat-maize crop combination. In soils that had been impaired by erosion, it also performed better at restoring crop yield (Jadoon et al. 2003)). In a field environment, consideration is given to the phosphorus content of maize derived from organic manures, such as crop residue, bio-organic matter, chicken manure, and pressing mud. Researchers discovered that maize includes a significantly high amount of phosphorus (42.68%) compared to other sources, such as poultry manure. When utilized as a source of phosphorus, farmyard manure is substantially more successful than other treatments at boosting the accessibility of phosphorous from soil (Meena et al. 2007). The output of maize grain varies substantially as a result of the addition of organic manures. Although grain production for manure was equal to or, on occasion, greater when phosphorus was obtained from inorganic sources, applying poultry manure produces the highest grain output when compared to another method (Jama et al. 2007).

When applied in place of mineral fertilizers, organic manures increase the grain production of maize (Reddy et al. 2007). Compared to other organic sources, poultry dung offers greater phosphorus that is readily available (Garg et al. 2007). Organic manure sources enhance the accessibility of organic matter, phosphorous, and potassium, as well as plant uptake of these nutrients. Organic amendments also enhance the amount of P and K in the overall plant, as is the height of the plant, LAI, dry matter manufacturing, and LAI. When it comes to plant P and K concentrations, poultry dung offers the best benefits. According to research, increasing the availability of nutrients in the soil from organic manures is what causes improved soil concentrations of nutrients and plant growth (Adnan 2021).

Somalia's agricultural sector faces several challenges, such as low soil fertility, inadequate access to modern farming techniques, and reliance on traditional farming practices. Due to these factors, yields of crops have remained low, and the nutritional security of the population has declined. Organic fertilizers may be used to surmount these obstacles and improve agricultural output in Somalia. However, more study is needed to determine the effectiveness of organic fertilizers in Somalia and how they affect crop productivity. Little is known and appreciated about organic fertilizers among Somali farmers. The vast majority of farmers in Somalia still use outdated methods and need more access to education and training in contemporary agriculture. Farmers are also not fully informed about the benefits of organic fertilizers and how to use them due to a lack of agricultural extensions and instructional programs. Due to growers' inadequate knowledge and understanding of organic fertilizers, their potential impact on crop output may be restricted, given how seldom they are now employed. The availability of organic fertilizers is a benefit of their use. Organic fertilizers could be hard to come by in Somalia, and farmers might not have the resources or expertise to create them themselves. This might promote the use of costly chemical fertilizers that harm the environment. Due to their limited accessibility and availability, organic fertilizers are challenging to utilize and may not have a significant impact on crop output. The unique climate and soil of Somalia may affect the efficacy of organic fertilizer in addition to challenges with knowledge and accessibility. The effectiveness of organic fertilizers may vary depending on factors such as soil pH, nutrient content, and rainfall patterns.

This research aims to investigate how organic fertilizer affects crop production in Afgoi District-lower Shebelle, Somalia. The general purpose of this study is to identify the role of Organic fertilizer on crop production at Afgoi District-lower Shebelle, Somalia. To Identify the role of green manure on crop production at Afgoi District-lower Shebelle, Somalia. To Determine the role of animal manure on crop production at Afgoi District-lower Shebelle Somalia.

MATERIAL AND METHOD

The research approach of the study was the main focus of this chapter. The chapter includes research design, target population, sample size, sampling procedures, research instruments, and Research quality involves collecting the data, collecting the data, interpreting the data, and finally limiting the study while addressing ethical considerations.

Research Design

The study was conducted using a descriptive design. In order to discuss and examine the impact organic fertilizer has on crop production in Somalia's Afgoi District Lower Shebelle. Utilizing A data obtained from the questionnaire. The target population can be easily reached, the design is efficient, and it is less expensive, all of which make it the best option for this study.

Research Population

The target population of the study was 400 from Afgoi District- lower Shebelle, Somalia.

Sample Size

The researchers used Solvent's formula to calculate the sample size, with a maximum acceptable error of 5%.

$$n = \frac{N}{1+Ne^2}$$

N: Stands for the population

n. Stands for the sample size

e. Stands for acceptable error

n= $\frac{400}{1+400(0.05)^2}$= so the sample was 200

Sampling Procedure

The sampling method was non-random sampling anon-simple random sampling was selected to collect information from the accessible populations of farmers, Agro-students, and workers.

This study's findings about the role of organic fertilizer on crop production have been established in a number of ways. The research's conclusions and findings from a structured questionnaire are discussed in this section. This study's overarching goal is to determine how organic fertilizer affects crop output in Somalia's Afgoi District (lower Shebelle). In contrast, the objective of the study is to identify the role of green manure on crop production at Afgoi District-lower Shabelle, Somalia. to determine the role of animal manure on crop production at Afgoi District-lower Shebelle, Somalia.

RESULTS AND DISCUSSION

The Role of Organic Fertilizers

It is known that soil quality and productivity are increased with organic fertilizer application. For this purpose, many organic fertilization materials are widely used (Figure 1). According to the study's findings, the majority of participants (56%) indicated high agreement that green manure improves crop output by directly aerating soil fertility. In contrast, the majority of respondents (47.5%) thought that plants grown in green manure receive nutrients from deeper soil layers and leave them in the topsoil. The majority of responders (54%) strongly disagree, however, that green manure enhances the availability of both macro and micronutrients. Most poll respondents (51%) completely agree that green manures are environmentally favorable in other contexts. Additionally, the majority of respondents (57.5%) firmly concur that green manure raises the standard of produce and crops.

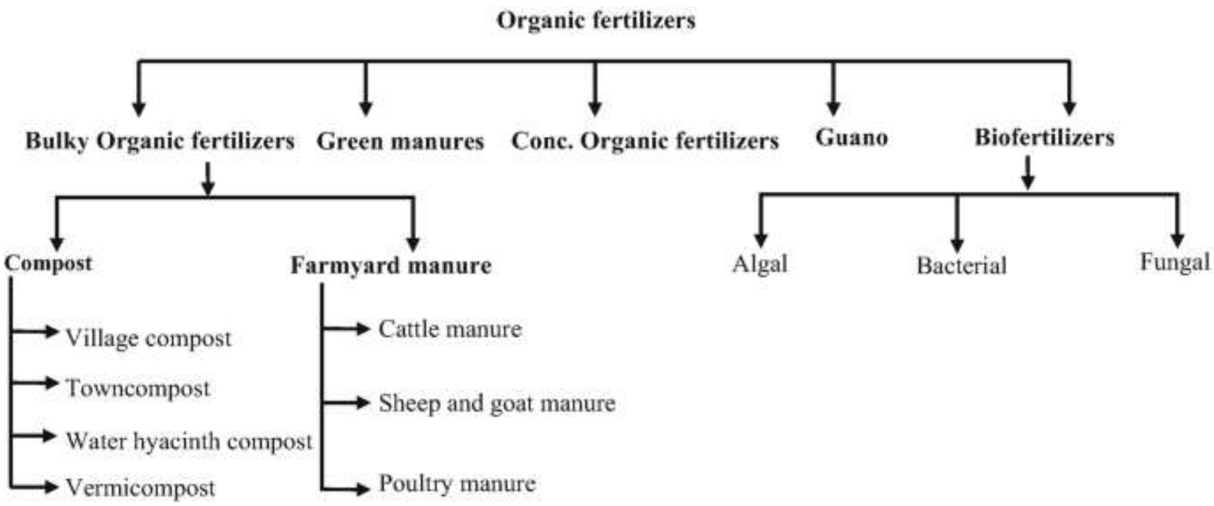


Figure 1. Organic fertilizers used in improving soil fertility (Singh et al. 2020)

Animal Manure

The majority of respondents (51.5%) answered that they strongly agreed with the claim that spreading manure to croplands can help maintain or raise the level of soil organic matter. On the other hand, a large majority of the participants (38%) said they completely concurred with the assertion that animal dung from locally accessible sources contains both micro and macro nutrients needed for crop productivity in organic as well as inorganic forms.

Crop Production

Organic fertilizers are among the most important inputs in plant production. In fact, it is reported that organic fertilizers can be a good alternative in combating stress factors in plant production (Figure 2).

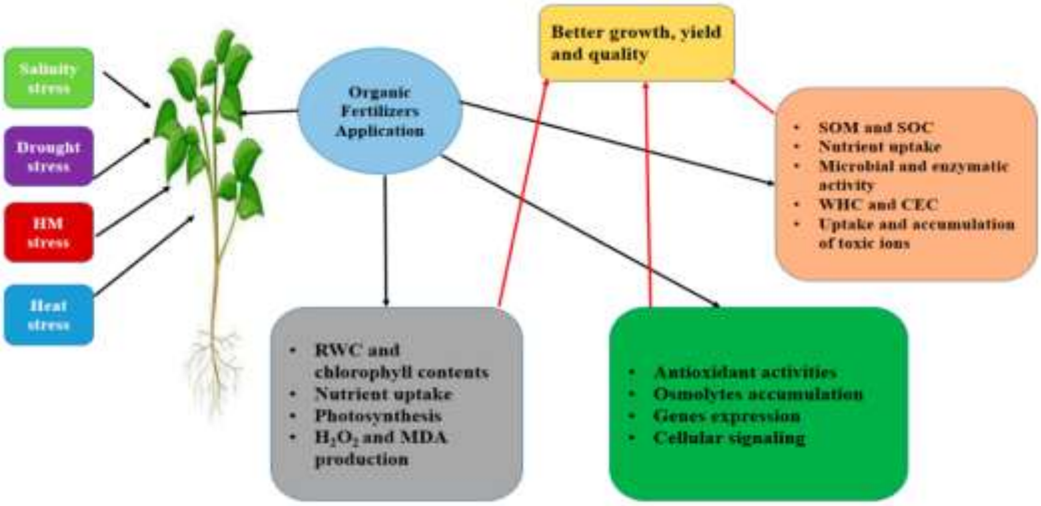


Figure 2. Effects of organic fertilizer application against stress factors in plant production (Liu et al. 2024)

The majority of respondents (55%) said firmly that crop production is the primary means of subsistence for Afgoi communities, according to the data. Conversely, most respondents (37.5%) believed that the production system had improved during the previous two years. Alternatively, the majority of responses (49.5%) agreed that the application of organic fertilizer increases crop productivity per unit of surface area. In a similar vein, the majority of responders (52.5%) strongly concur that fertilizer quality affects crop productivity.

With regard to the result of this study and based on its objectives, the following could be concluded: The objective of the role of Organic fertilizer on crop production indicates that the use of green manure is widely believed to increase soil fertility through the direct addition of nitrogen, as well as by absorbing nutrients from deeper soil layers and leaving them in the surface soil. Respondents also strongly agree that green manure increases the availability of both macro and micronutrients, is environmentally friendly, and improves the quality of crops and produce.

The objective of the role of animal manure on crop production shows that the use of animal manure can help maintain or improve soil organic matter levels and that it contains both macro- and micronutrients needed for crop production in organic and inorganic forms.

Respondents also strongly agree that animal manure from locally available sources is cheaper than inorganic fertilizers and that the best animal manure is from poultry.

Crop productions conclude that crop production is the livelihood strategy for Afgoi communities, with most respondents strongly agreeing with this statement. While a smaller percentage of respondents agreed that the production system has improved in the past two years, the use of organic fertilizers is widely believed to increase crop production per unit of surface area. Respondents also strongly agree that the quality of fertilizers is an important factor in crop production.

CONCLUSIONS

To improve crop production and soil fertility, increase the application of organic fertilizers like green manure and animal faces. To educate farmers about the advantages and appropriate methods for the application of organic fertilizers, services for extension should be made available. Develop and implement operational policies that encourage the creation and application of organic fertilizers. In order to assure the safety and efficacy of organic fertilizers, restrictions and subsidies for their production and distribution may be necessary.

Encourage the use of locally accessible sources of animal dung, particularly poultry manure, as a reasonably priced substitute for inorganic fertilizers. This can entail offering instruction and assistance for small-scale chicken farming. Increase funding for the production of organic fertilizers and the technologies used to apply them. This could result in new, enhanced organic fertilizers that are more successful and efficient at enhancing crop production and soil fertility. Encourage and support farmer cooperatives and associations to promote the use of organic fertilizers and improve crop production. This could include providing access to credit and market information and facilitating collective bargaining for better prices and market opportunities.

REFERENCES

Adnan, M. (2020). Remote Sensing an Innovative Way to Improve Crop Production: A Review. *Current Trends in Engineering Science (CTES), 1*(1), 36–38. https://www.researchgate.net/publication/344886862

Adnan, M., & Asif, M. et al. (2020). Organic and Inorganic Fertilizer; Integral Part for Corp Production. *EC Agriculture, 6*(3), 1–7.

Antil, R. S., & Singh, M. (2007). Effects of organic manures and fertilizers on organic matter and nutrient status of the soil. *Archives of Agronomy and Soil Science*, *53*(5), 519–528. https://doi.org/10.1080/03650340701571033

Assefa, S., & Tadesse, S. (2019). The Principal Role of Organic Fertilizer on Soil Properties and Agricultural Productivity -A Review. *Agricultural Research & Technology: Open Access Journal*, *22*(2), 1–5. https://doi.org/10.19080/ARTOAJ.2019.22.556192

Bergstrand, K. J. (2022). Organic fertilizers in greenhouse production systems – a review. *Scientia Horticulturae*, *295*(November 2021), 110855. https://doi.org/10.1016/j.scienta.2021.110855

Bilen, E. (2008). *Evaluation of pre-crops and fertilization on organic zucchini under the Mediterranean condition: A case study*. 6–7.

Cai, A., Xu, M., Wang, B., Zhang, W., Liang, G., Hou, E., & Luo, Y. (2019). Manure acts as a better fertilizer for increasing crop yields than synthetic fertilizer does by improving soil fertility. *Soil and Tillage Research*, *189*(February 2018), pp. 168–175. https://doi.org/10.1016/j.still.2018.12.022

De Ponti, T., Rijk, B., & Van Ittersum, M. K. (2012). The crop yield gap between organic and conventional agriculture. *Agricultural Systems*, *108*,1–9. https://doi.org/10.1016/j.agsy.2011.12.004

Doran, J. W., & Smith, M. S. (2015). *Organic Matter Management and Utilization Of Soil and Fertilizer Nutrients*. *19*, 53–72. https://doi.org/10.2136/sssaspecpub19.c4

Getu, A. (2015). Effect of adaptable green manure plants on sorghum yields and soil fertility in Eastern Amhara Region of Ethiopia. *Journal of Biology, Agriculture and Healthcare*, *5*(11), 223–231. http://www.iiste.org/Journals/index.php/JBAH/article/view/23256/23712

Hossain, M., Shamsuddoha, A., Paul, A., Bhuiyan, M., & Zobaer, A. (1970). Efficacy of Different Organic Manures and Inorganic Fertilizers on the Yield and Yield Attributes of Boro Rice. *The Agriculturists*, *9*(1–2), pp. 117–125. https://doi.org/10.3329/agric.v9i1-2.9486

Ilahi, H. (2021). Accentuating the Impact of Inorganic and Organic Fertilizers on Agriculture Crop Production: A Review. *Indian Journal of Pure & Applied Biosciences*, *9*(1), 36–45. https://doi.org/10.18782/2582-2845.8546

Jiang, G., Zhang, W., Xu, M., Kuzyakov, Y., Zhang, X., Wang, J., Di, J., & Murphy, D. V. (2018). Manure and Mineral Fertilizer Effects on Crop Yield and Soil Carbon Sequestration: A Meta-Analysis and Modeling Across China. *Global Biogeochemical Cycles*, *32*(11), 1659–1672. https://doi.org/10.1029/2018GB005960

Khosro, M., & Yousef, S. (2012). Bacterial Biofertilizers for Sustainable Crop Production : a Review. *Journal of Agricultural and Biological Science*, *7*(5), 307–316.

Liu Y, Lan X, Hou H, Ji J, Liu X, Lv Z. (2024). Multifaceted Ability of Organic Fertilizers to Improve Crop Productivity and Abiotic Stress Tolerance: Review and Perspectives. *Agronomy* 14(6):1141. https://doi.org/10.3390/agronomy14061141

Meena, S. L., Lakhera, J. P., Sharma, K. C., & Johri, S. K. (2012). Knowledge Level and Adoption Pattern of Rice Production Technology Among Farmers. *Rajasthan Journal of Extension Education*, *20*(3), 133–137. http://www.rseeudaipur.org/wp-content/uploads/2013/02/323.pdf

Mishra, D., Rajvir, S., Mishra, U., & Kumar, S. (2013). Role of Bio-Fertilizer in Organic Agriculture: A Review. *Research Journal of Recent …*, *2*(2013), 39–41. http://isca.in/rjrs/archive/special_issue2012/8.ISCA-ISC-2012-1AFS-32.pdf

Mousavi, S. R., Galavi, M., & Rezaei, M. (2013). Zink (Zn) Importance for Crop Production. *International Journal of Agronomy and Plant Production*, *4*(1), 64–68.

Parr, J. F., Hornick, S. B., & Kaufman, D. D. (1994). Use of Microbial Inoculants and Organic Fertilizers in Agricultural Production. *Soil - Microbial Systems Laboratory*, *11*(01), 1–15.

Power, J., & Prasad, R. (1997). Organic Manures. *Soil Fertility Management for Sustainable Agriculture*, 323–337. https://doi.org/10.1201/9781439821985.ch19

Rana, J., & Paul, J. (2017). Consumer behaviour and purchase intention for organic food: A review and research agenda. *Journal of Retailing and Consumer Services*, *38*(February), 157–165. https://doi.org/10.1016/j.jretconser.2017.06.004

Riahi, A., Hdider, C., Sanaa, M., Tarchoun, N., Kheder, M. Ben, & Guezal, I. (2009). The influence of different organic fertilizers on yield and physicochemical properties of organically grown tomato. *Journal of Sustainable Agriculture*, *33*(6), 658–673. https://doi.org/10.1080/10440040903073800

Senesi, N. (1989). Composted materials as organic fertilizers. *Science of the Total Environment, The*, *p. 81–82*(C), pp. 521–542. https://doi.org/10.1016/0048-9697(89)90161-7

Sharma, A. (2017). A Review on the Effect of Organic and Chemical Fertilizers on Plants. *International Journal for Research in Applied Science and Engineering Technology*, *V*(II), pp. 677–680. https://doi.org/10.22214/ijraset.2017.2103

Singh, T.B. *et al.* (2020). Role of Organic Fertilizers in Improving Soil Fertility. In: Naeem, M., Ansari, A., Gill, S. (eds) Contaminants in Agriculture. Springer, Cham. https://doi.org/10.1007/978-3-030-41552-5_3

Soane, B. D. (1990). The role of organic matter in soil compactibility: A review of some practical aspects. *Soil and Tillage Research*, *16*(1–2). 179–201. https://doi.org/10.1016/0167-1987(90)90029-D

Stein-Bachingerxy, K., & Werner, W. (1997). Effect of Manure on Crop Yield and Quality in an Organic Agricultural System. *Biological Agriculture and Horticulture*, *14*(3), 221–235. https://doi.org/10.1080/01448765.1997.9754812

Suge, J. K., Omunyin, M. E., & Omami, E. N. (2011). Effect of organic and inorganic fertilizer sources on growth, yield and fruit quality of eggplant (Solanum Melongena L). *J.K. Suge et Al Arch. Appl. Sci. Res*, *3*(6), 470–479.

Thomas, C. L., Acquah, G. E., Whitmore, A. P., McGrath, S. P., & Haefele, S. M. (2019). The effect of different organic fertilizers on yield and soil and crop nutrient concentrations. *Agronomy*, *9*(12). https://doi.org/10.3390/agronomy9120776

Zahangir Hossain, M., von Fragstein, P., & Heß, J. (2016). Plant Origin Wastes as Soil Conditioner and Organic Fertilizer: A Review. *& Environ. Sci*, *16*(7), 1362–1371. https://doi.org/10.5829/idosi.aejaes.2016.16.7.12961

Zelleke, G., Agegnehu, G., Abera, D., & Rashid, S. (2010). Fertilizer and Soil Fertility Potential in Ethiopia Table of Contents. *Fertilizer and Soil Fertility Potential in Ethiopia*, *July*, 1–66.

APPROACHES TO OBTAINING OPTIMUM BENEFIT FROM MICROBIAL PRODUCTS USED IN PLANT PRODUCTION

Ahmet Cemil EKEN[1], İsmail Emrah TAVALI[1], Hüseyin OK[2]
[1] Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Akdeniz University, 07059 Antalya, Türkiye
[2] Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Cyprus International University, 99258 Nicosia, Türkiye

Correspondence: etavali@akdeniz.edu.tr

ABSTRACT

It is clear that in order to achieve higher and better-quality yields per unit area, it is not enough to only focus on plant breeding; effective plant nutrition through adequate watering and proper fertilization (based on the plant's phenological stage and in appropriate amounts) is crucial for obtaining the desired results. Growing healthy, high-yielding, and high-quality crops primarily involves proper soil management. In this context, a plant grown in soil with low organic matter levels (below 2%) and consequently reduced microbial diversity will struggle to cope with disease agents (especially soil-borne ones), have low vegetative and generative organ development, and ultimately may not benefit significantly from the minerals present in the soil or added through fertilizers. In addressing this common issue, commercial products containing microorganisms with functional benefits for soil and plants (such as bacteria, fungi, algae, etc.) which can aid in plant nutrition, plant protection, and plant growth regulation represent a significant alternative to chemical inputs (such as chemical fertilizers, pesticides, and synthetic growth regulators). However, to maximize the benefits of these products, it is essential to determine not only their capabilities under laboratory conditions (such as nitrogen fixation, phosphorus solubilization, siderophore production, auxin production, etc.) but also their abilities to colonize plant roots, proliferate in the rhizosphere, and compete in various plant and soil types under greenhouse or field conditions. Moreover, factors such as the type of plant production area (field, garden, greenhouse), production model (conventional, organic, ecological, etc.), plant type (cereal, vegetable, fruit), irrigation method (surface irrigation, pressurized irrigation), and especially soil characteristics (texture, organic matter, lime, pH, etc.) must be considered when selecting the appropriate product(s).

Keywords: Biofertilizer, Biological agent, Biostimulant

INTRODUCTION

Fertilizer is one of the inputs used to ensure the continuity of food and agricultural products necessary to meet basic needs such as nutrition and shelter, which have increased due to the growing population and steadily rising living standards. Ensuring the productivity and sustainability of agricultural lands is possible by returning the plant nutrients lost from the soil due to various reasons back to the soil (TAGEM 2018).

Fertilizers used to increase agricultural production and product quality are generally classified into two categories in the agricultural sector: chemical fertilizers and organic fertilizers (Akmermer 2022). The nutrient content of organic fertilizers is significantly lower compared to chemical fertilizers and is not in a form that plants can directly benefit from. For this reason, organic fertilizers are used for soil regulator purposes rather than meeting the nutritional needs of plants (Yetgin 2010). Chemical fertilizers, on the other hand, typically contain plant nutrients in a form that can be readily absorbed by plants and in high concentrations, allowing nutrient deficiencies to be addressed quickly and easily, with lower costs and labor (Evni ve Kan 2024).

Chemical fertilizers, generally produced as nitrogenous, phosphorous, and potassium-based, play a significant role in increasing crop productivity. However, the intensive use of chemical fertilizers not only enhances crop yields but also brings certain disadvantages. Chemical fertilizers alter the physical, chemical, and biological properties of the soil. Continuous use of chemical fertilizers leads to a decline in soil quality, including a reduction in soil organic matter (SOM) content. Excessive use of chemical fertilizers hardens the soil, reduces soil fertility, pollutes the air, water, and soil, and significantly decreases the availability of soil minerals, thereby posing environmental hazards. The continuous application of chemical fertilizers changes the soil pH, potentially increasing acidity. This can lead to a reduction in beneficial organisms, hinder plant growth and even contribute to an increase in greenhouse gas emissions due to the decrease in organic matter (Pahalvi vd. 2021).

In 2021, nitrogenous fertilizers accounted for most of the global fertilizer production, with a volume of 118.55 million tons, representing 55.53% of the total 213.46 million tons produced worldwide (FAO 2024). Regarding consumption, as shown in Figure 1, it was reported that by 2022, 109.29 million tons of nitrogenous fertilizers, 44.1 million tons of phosphorous fertilizers, and 34.66 million tons of potassium fertilizers were used worldwide (IFASTAT 2024).

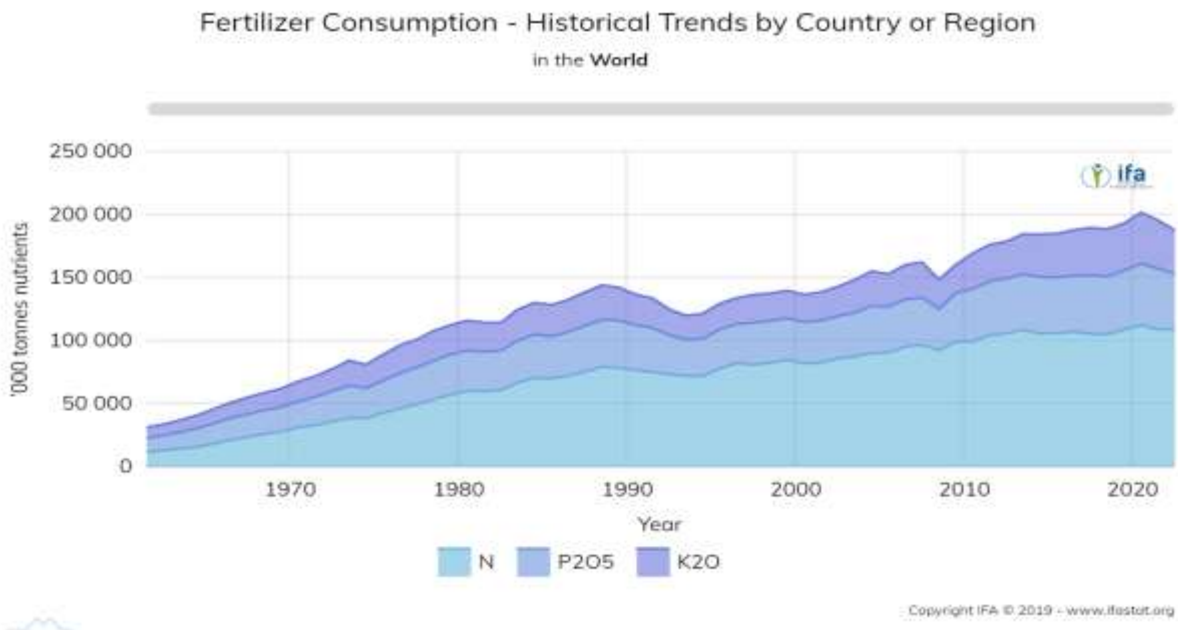


Figure 1. Global consumption amounts of N, P_2O_5, and K_2O fertilizers by year (IFASTAT 2024)

Fertilizer production worldwide is shaped regionally according to consumption volumes and raw material resources. Countries with large populations, such as the USA, China, India, Canada and Russia also have extensive agricultural lands, giving them a significant share in fertilizer usage (Figure 2). These countries are also major fertilizer producers (Kocagöz 2022).

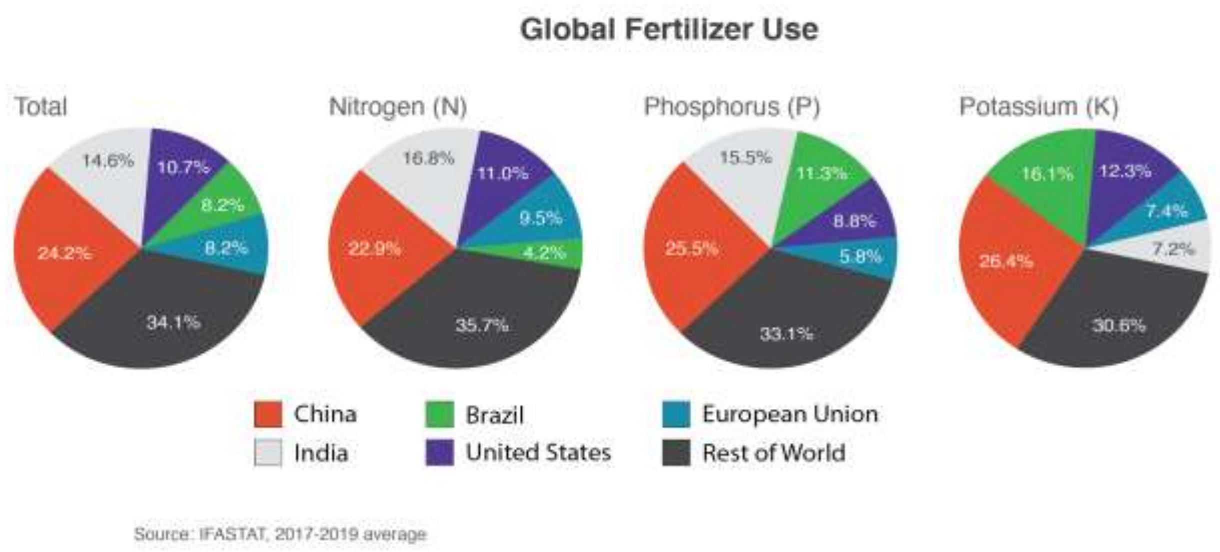


Figure 2. Fertilizer usage percentages in various countries worldwide

One of the most significant issues in the fertilizer sector is the dependence on imports for countries lacking raw materials. The supply of essential inputs, such as natural gas and phosphate rock is largely reliant on external sources. This situation makes fertilizer production companies heavily dependent on imports, which can lead to price fluctuations and increases, as well as challenges in ensuring a continuous supply of high-quality raw materials. Additionally, this dependency, coupled with factors such as raw material-producing countries entering the fertilizer production market, intensifies competition. As a result, the global fertilizer market is dominated by a few countries, creating an oligopolistic market structure (TAGEM 2018).

APPLICATION OF MICROBIAL PRODUCTS IN PLANT PRODUCTION

Given the environmental threats associated with the use of chemical fertilizers and considering the high consumption volumes and associated costs for countries lacking raw materials, there is a clear need for alternative solutions due to the economic disadvantages. One of the primary alternative solutions is microbial products, which are directly related to soil organic matter.

With changing climate and ecosystems, biological approaches aimed at improving plant production continue to gain strong support among engineers and academics focused on sustainability in agriculture. In this context, there is a growing global momentum in exploring a wide variety of rhizobacteria with new properties, such as heavy metal detoxification potential (Ma et al. 2011; Wani and Khan 2010). The rhizosphere, the soil region surrounding plant roots, represents the area of intense microbial activity in the soil (Cantó et al. 2020). Biological fertilizers, also known as bio-fertilizers, are materials composed of living microorganisms that can colonize the rhizosphere and/or penetrate plant tissues. When applied to the plant surface or soil, they fix free nitrogen, process mineral elements from organic and inorganic sources to increase their availability and uptake by plants, and/or stimulate optimal plant growth through the production of secondary metabolites (Çakmakçı 2005). Both prokaryotes (archaea, bacteria and viruses) and eukaryotes (fungi, oomycetes, nematodes, protozoa, algae and arthropods) colonize the rhizosphere. Among these, bacteria and fungi are the most prevalent in terms of population (Buee et al. 2009).

Soil-dwelling microorganisms that enhance plant growth are bacteria living on or around the root surface. They contribute to plant growth and development directly or indirectly by producing and secreting various regulatory chemicals in the rhizosphere. Beneficial microorganisms can help reduce global reliance on chemical fertilizers in agriculture (Ahemad and Kibret 2014). They increase the availability of nutrients such as phosphorus (P), potassium (K), zinc (Zn), selenium (Se), and iron (Fe) in the soil through biochemical processes like dissolution, chelation, mineralization, oxidation, and reduction reactions. Additionally, microorganisms are known to secrete phytohormones, including auxins, cytokinins, abscisic acid, ethylene, brassinosteroids, jasmonic acid, salicylic acid, strigolactones, and gibberellins, which act as plant growth regulators and stress mitigators (Koskey et al. 2021). Various studies have shown that applying rhizobacteria, which promote plant growth under both normal and stressful conditions such as salinity, drought, acidity, or alkalinity, can improve the health and productivity of different plant species (Ahemad and Kibret 2014).

Indirect effects of microbial products include enhancing plant health by suppressing phytopathogens and other harmful microorganisms through parasitism, creating competitive environments for nutrients in the rhizosphere, and producing antagonistic substances such as siderophores, antibiotics, and antimicrobial metabolites. Additionally, these microorganisms generate lytic enzymes like chitinase, glucanase, and protease (Figure 3). Some bacterial genera have been shown to protect plants from fungal and bacterial pathogens. These bacteria employ various mechanisms to inhibit the development of plant pathogens. These mechanisms include colonizing infection sites, competitively excluding pathogens, engaging in antagonistic activities through the secretion of antibiotics and lytic enzymes, and enhancing plant resistance (Bonaterra et al. 2022).

Benefits of PGPR Inoculation to Plants	PGPR Strain(s)	Tested Plant(s)
Tolerance to drought stress	*Pseudomonas fluorescens* DR11, *Enterobacter hormaechei* DR16, *Pseudomonas migulae* DR35, *Bacillus subtilis*, *Achromobacter piechaudii* ARV8, *Phyllobacterium brassicacearum*, *Paenibacillus polymyxa*, *Rhizobium tropici*, *Azospirillum brasilense*	Foxtail millet (*Setaria italica* L.), Maize (*Zea mays* L.), Bean (*Phaseolus vulgaris* L.), *Arabidopsis thaliana*, Tomato (*Lycopersicum esculentum* Mill cv. F144), Pepper (*Capsicum annuum* L. cv. Maor), Wheat (*Triticum aestivum* L.)
Tolerance to salinity stress	*Bacillus pumilus*, *Exiguobacterium oxidotolerans*, *Bacillus megaterium*, *Azospirillum* sp., *Achromobacter piechaudii*, *Eneterobacter* sp. PR14	Brahmi (*Bacopa monnieri* L.), Maize (*Zea mays* L.), Lettuce (*Lactuca sativa* L.), Tomato (*Lycopersicum esculentum* Mill.), Rice (*Oryza sativa* cv. Sahbhagi), Sorghum (*Sorghum bicolor*), Finger Millets (*Eleusine coracana*)
Tolerance to biotic stress (biocontrol)	*Paenibacillus xylanexedens*, *Bacillus amyloliquefaciens*, *Streptomyces* sp., *Ochrobacttrum intermedium*, *Paenibacillus lentimorbus*, *Pseudomonas* spp.	Wheat (*Triticum aestivum* L.), Rice (*Oryza sativa*), Pine (*Pinus taeda* L.), Tomato (*Lycopersicum esculentum* Mill.)
Increased nutrient absorption	*Pantoea* sp. S32, *Paenibacillus polymyxa*	Rice (*Oryza sativa* L.), Habanero pepper (*Capsicum chinense*)
Seed germination enhancement	*Serratia marcences*, *Pseudomonas fluorescens*, *Azospirillum lipoferum*, *Pseudomonas putida*, *Bacillus subtilis*, *Providencia* sp., *Brevundimonas diminuta*	Maize (*Zea mays* L.), Wheat (*Triticum aestivum* L.)
Biostimulation by phytohormone(s) production	*Azospirillum lipoferum*, *Bacillus subtilis*, *Arthrobacter protophormiae*, *Dietzia natronolimnaea*, *Bacillus* sp.	Rice (*Oryza sativa* L.), Tomato (*Solanum lycopersicum* L.), Wheat (*Triticum aestivum* L.)
Soil fertility enhancement	*Bacillus subtilis*, *Bacillus cereus*, *Rhizobium* spp.	Poplar (*Populus* sp.), Mung bean (*Vigna radiata* L.)
Bioremediation of heavy metals and pollutants	*Ochrobactrum* sp., *Bacillus* spp., *Pseudomonas* spp., *Pseudomonas fluorescens*, *Bacillus cereus*, *Alcaligenes feacalis* RZS2, *Pseudomonas aeruginosa* RZS3, *Enterobacter* sp. RZS5	Rice (*Oryza sativa* L.), Groundnut (*Arachis hypogaea*), Maize (*Zea mays* L.), Ashwagandha (*Withania somnifera*)
Modulation of plant secondary metabolites	*Bacillus subtilis*, *Azotobacter chroococcum*, *Pseudomonas putida*, *Bacillus pumilus*, *Exiguobacterium oxidotolerans*	Basil (*Ocimum basilicum*), Brahmi (*Bacopa monnieri* L.)

Figure 3. An overview of the mechanisms of microorganisms in inoculated plants (Basu et al., 2021).

NEW APPROACHES IN THE USE OF MICROBIAL PRODUCTS

While live microorganisms aim to provide beneficial plant nutrition and health properties, it is important to consider the factors that may limit these benefits. Microorganisms that exhibit positive characteristics under laboratory conditions may not always deliver the desired benefits in natural environments due to various environmental factors. Especially, when dealing with disease agents, it is crucial to thoroughly analyze the relationships between plants and pathogens, as well as the prevalent environmental factors in a specific area, before implementing biological control measures. Instead of relying on a single microorganism, applying a population of microorganisms with cooperative traits is more beneficial, as it creates a stable rhizosphere that provides more effective control against pathogens. In addition to microbial applications, the use of other plant products, such as extracts, biofertilizers and biopesticides, which are natural enemies of pests and/or disease-causing pathogens, also contributes to the success of biological control (Pandit et al. 2022).

In soil, only about 5% of the necessary nutrients are available compared to those in laboratory conditions. Additionally, there is ongoing competition for essential nutrients like nitrogen and carbon, meaning only microorganisms with strong competitive abilities can survive (Shah et al. 2021). Climate change is contributing to a steady increase in soil temperatures (Zhang et al. 2019). This rise impacts microorganisms closely associated with plants, as their optimal growth, diversity, and physiological functions depend on specific temperature ranges (Wu et al. 2010). Deviations from these optimal temperatures can lead to varied outcomes in microorganism-plant interactions. Soil warming notably increases microbial respiration and mortality rates (Wu et al. 2010; Schindlbacher et al. 2011). Moreover, excessive water evaporation and the accumulation of chloride salts, such as sodium chloride (NaCl) and magnesium chloride ($MgCl_2$), raise soil salinity. This increase in salt concentration adversely affects agricultural microbial biomass, seed germination, and plant development by disrupting osmotic potential and ion exchanges (Sindhu et al. 2022).

Host plant and microorganism interactions often determine host specificity, which can lead to a decrease in microbial benefit rates in many crop plant species. In soils with microbial diversity, the lack of specificity in plant species can lead to high competition among microorganism populations (Figueiredo et al. 2011). Additionally, any changes in biochemical compounds produced by plant roots or signaling molecules produced by microorganisms can disrupt potential symbiotic relationships (Chagas et al. 2018). Another important factor is soil pH. The availability of nutrients in the soil solution, for both plants and microorganisms, is greatly influenced by soil pH. The pH is a measure of the hydrogen ion concentration in the colloidal soil solution and affects most chemical reactions in the soil. Acidic soils are dominated by *Acidobacteria*, while alkaline soils are dominated by *Actinobacteria*, causing shifts in microbial populations with changes in soil pH. This can lead to the failure of the desired population to perform its intended function in microbial product use (Shah et al. 2021). Many rhizobacteria exhibit optimal population and efficacy at a pH level of 6.0 (Roe et al. 1998).

The commercialization and application of biological fertilizers involving live microorganisms begins with a technical procedure to isolate ectophytic or endophytic microorganisms from the rhizosphere and plant tissues, especially the roots. This process starts with bioresearch, where plant or soil samples are collected from diverse environments and subjected to microbial isolation in the laboratory. Once rhizobacteria are isolated, they undergo in vitro screening to evaluate their potential for promoting plant growth or serving as biocontrol agents against phytopathogens. Promising strains demonstrating effective plant growth promotion or biocontrol properties are then tested under controlled environmental conditions to assess their impact on plant development. Effective strains are selected based on these tests and further evaluated under field conditions to determine their ability to enhance crop growth. After successful repeated trials, companies typically proceed to commercialize these biological strains as biological fertilizers and/or biocontrol products, following formulation of the final product (Nakkeeran et al. 2005).

An unconventional approach in the field of microbial products that has emerged due to advances in genetics involves enhancing microorganism species and their efficacy, as well as developing new variants. This approach involves intentionally modifying the genetic material of microorganisms, specifically DNA to improve the beneficial traits of microbial strains. Scientists can alter bacterial DNA using techniques such as CRISPR-Cas9, which allows for specific modifications. This genetic manipulation can help bacteria produce chemicals that assist in their proliferation, growth, stress resistance or more efficient nutrient utilization (Bernela et al. 2021; Hasan et al. 2024).

In recent years, leading global agricultural firms have increasingly focused their investments on bio-based product research. Figure 4 lists the revenues and profits of some international companies in the plant protection sector for the second quarter of 2024. Additionally, Figure 5 shows the targeted revenues from bio-based products and the budgets allocated for research and development (R&D) by these same firms for the year 2024. Comparing the data in both figures reveals that firms leading the world in pesticide production are allocating a significant portion of their current revenues to R&D projects aimed at generating revenues from bio-based products over the next decade.

Q2 2024 CROP PROTECTION COMPARISON

COMPANY	REVENUE	REVENUE CHANGE YOY (%)	EBITDA	EBITDA MARGIN (%)
BAYER	$5.42 Billion	1.1%	$572 million**	10.5%
Syngenta Group	NA	NA	NA	NA
CORTEVA	$6.11 Billion	1%	$1.92 Billion	29%
BASF We create chemistry	$2.11 Billion	13.2%	$147 million**	6.9%
FMC	$1.04 Billion	2%	$202 million	20%
UPL	$1.09 Billion	1%	$137.4 million	12.6%

2024 Q2 Numbers with USD conversion at 1.09 (UPL Q1 24/25)
**Before Special Items
Sources: Company Quarterly Reports
Red indicates lower than 2023

Upstream

Figure 4. Revenue Table of Some International Firms in the Plant Protection Sector (UPSTREAM 2024)

2024 AGRIBUSINESS BIO-BASED REVENUE COMPARISON

COMPANY	CURRENT BIO-BASED REVENUE	FORECASTED REVENUE	FORECASTED CAGR THROUGH PERIOD	2023 R&D EXPENDITURE*	PARTNERSHIPS, INVESTMENTS & ACQUISITIONS****
BAYER	$215 million (2022)	$1.61 billion by 2035	17%	$2.5 billion***	Sound, JOYN BIO, kimitec
syngenta	$400 million (2023)	NA	NA	$1.4 billion	Valagro, VESTARON, biotalys, azotic, lavie bio, Sound
CORTEVA	$420 million (2023)	$2 billion by 2035	17%	$1.34 billion	Symborg, Stoller, elemental enzymes, lavie bio
FMC	$180 million (2023)	$2 billion by 2033	20%	$329 million	AgroSpheres, micropep, novonesis
UPL	~$400 million (2022/23)**	NA**	14% through 2027	$195 million	Arysta

*2023 Numbers with USD conversion, except UPL 2022/23
**UPL has not release its 2023/24 results in detail as of June 27th 2024. UPL "NPP Business Unit not entirely made up of biologicals. Given destocking challenges, UPL numbers likely significantly down in 23/24 so refraining from calculating the CAGR from 22/23 numbers
*** Bayer Annual Report cite $1.9 Billion USD, but have a "special items" EBITDA adjustment
**** Not all encompassing
Note: BASF has not publicly shared any biological numbers so they have been excluded
Sources: Company Annual Reports

Upstream

Figure 5. Targeted Revenue from Bio-Based Products and R&D Budget Allocation of Some International Firms (UPSTREAM 2024)

CONCLUSIONS

In today's rapidly evolving climate conditions and the anticipated impacts in the coming years, factors such as rising temperatures and drought, excessive use of chemical fertilizers, salinity from intense evaporation, global warming and reduced water resources due to unregulated water consumption are critical issues affecting agricultural production. Additionally, changing ecosystems due to climate change, the evolution of harmful pathogens and pests and the resulting resistance changes in nature due to pest control measures are pressing and unresolved problems in agriculture. To address these widespread issues and enhance the sustainability of agricultural production, microbial products containing bacteria, fungi, algae and other microorganisms offer a promising alternative. These products provide functional benefits for soil and plants, including plant nutrition, plant protection, and growth regulation, as alternatives to chemical fertilizers and plant protection products.

Some technical details regarding the use of these products emerge as factors determining their effectiveness. Research and advancements in the field aimed at eliminating environmental factors that limit the usefulness of microorganisms are promising. Similar to breeding efforts for plants to achieve higher yields, resistance to diseases and pests and meeting market quality criteria such as color, shape, brightness and taste, the production of live microbial fertilizers and microbial breeding through new technologies that minimize the impact of environmental conditions on microorganisms have opened up new possibilities in the field. It is encouraging to see international pesticide-producing firms investing in scientific research in this area. Additionally, the progress made by some plant nutrition and protection companies in Turkey, which are exploring bio-based product research and commercial applications represents a positive development in agricultural practices.

REFERENCES

Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Journal of King saud University-science, 26(1), 1-20.

Akmermer, B. Sector, G. T. O. F., & TURKEY'S, I. E. O. (2022). Güncel Gelişmelerin Etkisinde Gübre Sektörü Küresel Ticareti ve Türkiye'nin Tarımsal Sürdürülebilirliğine Etkileri. *KAYES-2022*, S 136.

Basu, A., Prasad, P., Das, S. N., Kalam, S., Sayyed, R. Z., Reddy, M. S., & El Enshasy, H. (2021). Plant growth promoting rhizobacteria (PGPR) as green bioinoculants: recent developments, constraints, and prospects. *Sustainability*, *13*(3), 1140.

Bernela, M., Rani, R., Malik, P., & Mukherjee, T. K. (2021). Nanofertilizers: applications and future prospects. In *Nanotechnology* (pp. 289-332). Jenny Stanford Publishing.

Bonaterra, A., Badosa, E., Daranas, N., Francés, J., Roselló, G., & Montesinos, E. (2022). Bacteria as biological control agents of plant diseases. *Microorganisms*, *10*(9), 1759.

Buee, M., De Boer, W., Martin, F., Van Overbeek, L., & Jurkevitch, E. (2009). The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors.

Carla de La Fuente Cantó, C., Simonin, M., King, E., Moulin, L., Bennett, M. J., Castrillo, G., & Laplaze, L. (2020). An extended root phenotype: the rhizosphere, its formation and impacts on plant fitness. *The Plant Journal*, *103*(3), 951-964.

Chagas, F. O., de Cassia Pessotti, R., Caraballo-Rodríguez, A. M., & Pupo, M. T. (2018). Chemical signaling involved in plant–microbe interactions. *Chemical Society Reviews*, *47*(5), 1652-1704.

Çakmakçı, R. (2005). Bitki gelişimini teşvik eden rizobakterilerin tarımda kullanımı. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 36(1), 97-107.

Evni, G., & Kan, M., (2024). Tarımda Kullanılan Sıvı Organik ve Mineral Kaynaklı Gübre Piyasasının Değerlendirilmesi; Kırşehir İli Örneği. *Ahi Evran Akademi*, *5*(1), 1-12.

FAO, (2024). 21 Ağustos 2024, Erişim adresi https://www.fao.org/faostat/en/#data/RFB

Figueiredo, M. D. V. B., Seldin, L., de Araujo, F. F., & Mariano, R. D. L. R. (2011). Plant growth promoting rhizobacteria: fundamentals and applications. *Plant growth and health promoting bacteria*, 21-43.

Hasan, A., Tabassum, B., Hashim, M., & Khan, N. (2024). Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: A review. *Bacteria*, *3*(2), 59-75.

IFASTAT, (2024). 24 Ağustos 2024, Erişim adresi https://ifastat.org/databases/graph/1_1

Khanm, H., Vaishnavi, B. A., & Shankar, A. G. (2018). Raise of Nano-fertilizer ERA: Effect of nano scale zinc oxide particles on the germination, growth and yield of tomato (Solanum lycopersicum). *Int. J. Curr. Microbiol. Appl. Sci*, *7*(5), 1861-1871.

Kocagöz, Y. M. (2022). *Organik ve kimyasal gübre kullanımının karşılaştırılması: Tekirdağ ilinin Muratlı ilçesi örneği* (Master's thesis, Tekirdağ Namık Kemal Üniversitesi).

Koskey, G., Mburu, S. W., Awino, R., Njeru, E. M., & Maingi, J. M. (2021). Potential use of beneficial microorganisms for soil amelioration, phytopathogen biocontrol, and sustainable crop production in smallholder agroecosystems. *Frontiers in Sustainable Food Systems*, *5*, 606308.

Ma, Y., Rajkumar, M., Luo, Y., & Freitas, H. (2011). Inoculation of endophytic bacteria on host and non-host plants—effects on plant growth and Ni uptake. Journal of hazardous materials, 195, 230-237.

Nakkeeran, S., Fernando, W. D., & Siddiqui, Z. A. (2006). Plant growth promoting rhizobacteria formulations and its scope in commercialization for the management of pests and diseases. *PGPR: biocontrol and biofertilization*, 257-296.

Pahalvi, H. N., Rafiya, L., Rashid, S., Nisar, B., & Kamili, A. N., (2021). Chemical fertilizers and their impact on soil health. *Microbiota and Biofertilizers, Vol 2: Ecofriendly tools for reclamation of degraded soil environs*, 1-20.

Pandit, M. A., Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., ... & Kaur, J. (2022). Major biological control strategies for plant pathogens. *Pathogens*, *11*(2), 273.

Roe, A. J., McLaggan, D., Davidson, I., O'Byrne, C., & Booth, I. R. (1998). Perturbation of anion balance during inhibition of growth of Escherichia coli by weak acids. *Journal of bacteriology*, *180*(4), 767-772.

Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., & Zechmeister-Boltenstern, S. (2011). Experimental warming effects on the microbial community of a temperate mountain forest soil. *Soil Biology and Biochemistry*, *43*(7), 1417-1425.

Shah, A., Nazari, M., Antar, M., Msimbira, L. A., Naamala, J., Lyu, D., ... & Smith, D. L. (2021). PGPR in agriculture: A sustainable approach to increasing climate change resilience. *Frontiers in Sustainable Food Systems*, *5*, 667546.

Sindhu, S., Dahiya, A., Gera, R., & Sindhu, S. S. (2020). Mitigation of abiotic stress in legume-nodulating rhizobia for sustainable crop production. *Agricultural Research*, *9*(4), 444-459.

TAGEM (2018) Gübre Sektör Politika Belgesi 2018-2022. 20 Ağustos 2024, Erişim adresi https://www.tarimorman.gov.tr/TAGEM/Belgeler/yayin/Gübre%20Sektör%20Politika%20Belgesi%202018-2022.pdf

UPSTREAM, (2024). 25 Ağustos 2024. Erişim adresi https://www.upstream.ag/p/q2-2024-crop-protection-business

Wani, P. A., & Khan, M. S. (2010). Bacillus species enhance growth parameters of chickpea (Cicer arietinum L.) in chromium stressed soils. Food and Chemical Toxicology, 48(11), 3262-3267.

Wu, Y., Yu, X., Wang, H., Ding, N., & Xu, J. (2010). Does history matter? Temperature effects on soil microbial biomass and community structure based on the phospholipid fatty acid (PLFA) analysis. *Journal of Soils and Sediments*, *10*, 223-230.

Yetgin, M. A., (2010). Organik gübreler ve önemi. *Samsun Tarım İl Müdürlüğü Yayınları*.

Zhang, H., Liu, B., Zhou, D., Wu, Z., & Wang, T. (2019). Asymmetric soil warming under global climate change. *International journal of environmental research and public health*, *16*(9), 1504.

INVESTIGATION OF PECTIN AND XANTHAN GUM DEGRADATION BY INTESTINAL BACTERIA

Fatma CEBECİ[1],Tayyibe ERTEN[1]

[1] Bayburt University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Bayburt, Türkiye, 69000
Corresponding author e-mail: fatmacebeci@bayburt.edu.tr

ABSTRACT

The microbiota in the colon transforms polysaccharides such as pectin and xanthan gum and contributes to the maintenance of colon health by providing the formation of short-chain fatty acids. Various studies have shown that microbiota breaks down these components and form short-chain fatty acids, gas, and various metabolites, but in studies, the microbiota has generally been examined as a community, and the contribution of different bacterial species to biotransformation has not been sufficiently demonstrated. This study investigated whether *Escherichia coli* strains isolated from the human colon, which has not been studied before, produce short-chain fatty acids using pectin or xanthan gum in vitro. The results showed that when cultures containing different concentrations of polysaccharides were compared with control cultures (without polysaccharides), the lowest pH values were observed in cultures containing 5 mg/ml xanthan gum and 10 mg/ml pectin. However, gel formation was observed in the media prepared at these concentrations, and problems were experienced during the pipetting stages. For these reasons, 2 mg/ml xanthan gum and 5 mg/ml pectin concentrations were selected to test the production of short-chain fatty acids. The results of this study showed that these strains could not produce short-chain fatty acids under the conditions examined, but it aims to examine different bacterial strains in further studies.

Keywords: pectin, xanthan gum, microbiota, *E. coli*, short-chain fatty acids

INTRODUCTION

The human digestive system harbors a complex and dynamic population of microorganisms called the gut microbiota. This population of microorganisms includes bacteria, archaea, eukaryotes, and viruses (Koppel and Balskus, 2016). This population is larger than the number of human body cells, and it is estimated that there are at least 100 trillion (10^{14}) microorganism cells in the human gut (Clemente et al., 2012). The gut microbiota has a significant effect on the health and disease status of the host. The microbiota benefits the host by performing physiological functions such as strengthening intestinal integrity, shaping intestinal epithelial cells, obtaining energy, protecting against pathogens, and strengthening the host's immunity. Disturbance of the balance of the gut microbiota (dysbiosis) has been associated with the pathogenicity of many inflammatory diseases and infections (Thursby and Judge, 2017). The composition of the gut microbiota varies significantly among individuals. It is affected by many factors such as genetics, host physiology (age, disease, stress, etc.), medications used, diet, and living environment (Holscher, 2017). Although studies on the presence of microorganisms in placenta have been published in recent years (Aagard et al., 2014; Rodriguez et al., 2015), the common belief is that the intestinal microbiota begins to form at birth (Thursby and Judge, 2017). It is also known that antibiotic use in adulthood causes changes in the composition of the intestinal microbiota and that these changes can be long-term (Modi et al., 2014).

Diet is one of the important factors affecting the intestinal microbiota. There are many published studies, which indicates the influence of diet on microbiota. For instance, when the microbiota of people in a hunter-gatherer society in Tanzania was compared with those of people on a Western-style diet, it was found that the microbiota richness and diversity were higher in hunter-gatherers. This was associated with the fact that the diet of hunter-gatherers, unlike Western societies, included more carbohydrates that can be degraded by the microbiota (Schnorr et al., 2014). It is known that each of the macronutrients (fat, protein, carbohydrates) and many micronutrients can change the intestinal microbiota. The most prominent macronutrient among these is carbohydrates. For example, in a study examining fructose alone, it was found that it changed the composition of the intestinal microbiota (Mastrocola et al., 2018). An animal model study examining sucrose as part of a Western-style high-fat, high-sugar diet, it was observed that the microbiota was rapidly restructured (Collins et al., 2016). Furthermore, nutrients that humans cannot digest are used by bacteria in the large intestine in their basic biological processes. Therefore, changes in the human diet also affect the metabolism of these bacteria.

According to the Codex Alimentarius Commission (CAC) definition, dietary fiber is defined as carbohydrate polymers that are composed of 10 or more monomers and cannot be digested and absorbed in the human small intestine and is examined in 3 categories. These are classified as; edible carbohydrate polymers naturally found in foods; edible carbohydrate polymers obtained from raw food materials by physical, enzymatic or chemical means and having beneficial physiological effects based on scientific evidence; and finally, edible synthetic carbohydrate polymers having beneficial physiological effects based on scientific evidence. However, the definition of how many monomers dietary fiber will consist of may vary from country to country (Holsher, 2017). Dietary fibers can be obtained from plant and microbial sources. The main dietary fibers can be listed as: Cellulose, hemicellulose, guar gum, xanthan gum, alginate, carrageenan pectin, etc. (Guillon et al., 2011). These dietary fibers are broken down by intestinal bacteria when they reach the large intestine. As a result, they affect the stool mass, stool frequency, colon pH, and the energy obtained from these indigestible dietary fibers in individuals. However, the type of dietary fiber is broken down is important in terms of the metabolites formed (Guillon et al., 2011).

Epidemiological studies have shown that there is an inverse relationship between dietary fiber intake and cardiovascular diseases. The main important products formed as a result of the fermentation of these dietary fibers in the large intestine are short-chain fatty acids. The main short-chain fatty acids are acetate, propionate, and butyrate. Short-chain fatty acids play an important role in the health of the host. Butyrate contributes to the integrity of the intestinal barrier in the large and small intestines and provides energy to the epithelial cells in the large intestine (Chambers et al., 2018). In addition, studies have shown that butyrate has an inhibitory effect on colon cancer and the development of tumors. Different dietary fibers are formed with a wide variety of monosaccharide bonds. Although the human digestive system is inadequate in breaking down these bonds, the intestinal microbiota, with its diversity and gene pool, contains enzymes that can break down these bonds and can use unbreakable complex carbohydrates as an energy source (Gentile and Weir, 2018). Nutrition is one of the most important factors that shape and change the intestinal microbiota throughout human life. In addition, it is also a fact that the intestinal microbiota changes or transforms the foods consumed and the components in these foods. In this sense, the intestinal microbiota is one of the most important links in this bioavailability chain.

Pectin is one of the structural polysaccharides of plants and has a structure consisting of a polygalacturonic acid chain (Vandamme et al., 2002). It has a wide usage rate in the food industry due to its gelling, thickening and stabilizing properties. Since pectin can also be considered in the oligosaccharide class, it can also be fermented by intestinal bacteria (Willats et al., 2006). However, the rate of use by bacteria varies depending on the chemical structure and methylation degree of pectin (Hamaker et al., 2014). In some studies, it has been reported that pectic fractions support the development of bacteria such as *Bifidobacterium* and *Lactobacillus*, although it depends on the composition and prebiotic index of pectin. Thus, its benefits in protecting intestinal health and increasing digestion have been observed (Wicker et al., 2014). Gulfi and his colleagues (2007) determined that total short-chain fatty acid production was high as a result of fermenting pectin, which was extracted from apples and has a branched structure, with bacteria in human feces. In a study conducted with mice, Fåk and his colleagues (2015) observed that pectin increased the level of short-chain fatty acids in the cecum and blood, and stated that acetic acid production was low in low-methylation pectins.

Xanthan gum is a microbial polysaccharide produced by *Xanthomanas campestris*, which is widely used as a thickener and stabilizer in food, pharmaceutical and agricultural applications (Rosalam and

England, 2006). Since it is soluble in hot and cold water, it is used in many products in the food industry, and in the pharmaceutical industry, it is also used as a coating material with other polysaccharides such as chitosan (Giavasis, 2013). Xanthan gum, which is considered among the digestible carbohydrates, is also called dietary fiber and its use by intestinal bacteria was also investigated. In a study conducted by Bourquin et al., in vitro fermentation of various dietary fibers with bacteria isolated from feces was investigated, and they found that intestinal bacteria fermented xanthan and could produce high levels of acetic acid (Bourquin et al., 1996). Additionally, Schnizlein et al. (2020) found that in mice fed a diet supplemented with xanthan gum, the production of short-chain fatty acids increased and the efficacy of an antibiotic used to prevent colonization by *Colostroides difficile* bacteria was enhanced.
Studies showed that dysbiosis has been linked with many diseases. Various approaches, such as probiotics, prebiotic supplements, or fecal microbiota transplantation, have been intensively studied to cure the gut microbiota imbalance. Restoration of the levels of short-chain fatty acids is another therapeutic approach to cure dysbiosis (Fusco et al., 2023). Studies where the human microbiota was examined cumulatively showed that microbiota can degrade pectin and xanthan gum and can form acetate, propionate, and butyrate in different proportions. What has not been fully revealed in these studies and is worth examining is the contribution of individual members of the microbiota to this transformation. *Escherichia coli* is a bacterium commonly found in the human intestine. Previous studies have shown that *E. coli* strains can degrade different glucosides thanks to their different Glucosyl Hydrolase (GH) enzymes. In this context, this study investigated whether 2 different *E. coli* strains isolated from the human intestine can degrade pectin and xanthan gum and form short-chain fatty acids in vitro.

MATERIAL AND METHOD

E. coli strains used in the study were obtained from Karadeniz Technical University, Faculty of Medicine, Department of Microbiology. Nutrient Broth (Oxoid) was used to develop *E. coli* strains. After the liquid medium was prepared, the pH was adjusted to 6.8± 0.2. Pectin (pectin from Citrus peel, Galacturonic acid≥74.0) and xanthan gum (Xanthan from Xanthomonas campestris) used in the analyses were obtained from Sigma Aldrich.
The effect of different pectin and xanthan gum concentrations on the growth of *E. coli* strains were tested in the medium, 50 ml containing different concentrations of pectin (1, 2.5, 5, 10 mg/ml) and xanthan gum (0.5, 1, 2, 5 mg/ml). The media were inoculated with fresh *E. coli* F or *E. coli* C cultures and incubated for 8 hours in a shaking incubator (Heidolph, Germany) at 37°C. At the end of the incubation period, microbial growth in the cultures was determined by OD600 measurements made with a spectrophotometer (Thermo Scientific, Multiskan GO, US). The controls without pectin or xanthan gum were also prepared.
After determining the appropriate pectin and xanthan gum concentration, 50 ml media were prepared for each *E. coli* strain. These media were inoculated with fresh *E. coli* F or *E. coli* C cultures and incubated for 24 hours in a shaking incubator (Heidolph, Germany) at 37°C. At the end of the incubation period, pH measurements of the cultures were also made. In addition, fresh cultures of *E. coli* F and *E. coli* C were inoculated together on media containing pectin or xanthan gum (3 parallels, 50 ml medium). These media were incubated for 24 hours in a shaking incubator (Heidolph, Germany) at 37°C, and pH measurements were made at the end of the incubation period. After the prepared cultures were transferred to clean falcon tubes, they were stored at -20°C until analyzed.
GC-MS analysis was performed using the method Schneider et al. used (2006) with modifications. 0.4 ml of 50% sulfuric acid and 2 ml of diethyl ether were added to a sufficient amount (2 ml) of culture; the sample was mixed in an orbital shaker for 45 minutes and then centrifuged at 3000 rpm for 5 minutes. Potassium persulfate was added to remove the remaining water and injected into the device. Shimadzu GC-MS QP2010 Ultra and DB-FastFAME 30 m, 0.25 mm, 0.25 μm (Agilent, US) columns were used in the analyses. The temperature program

was programmed to increase from 100°C to 240°C and lasted for a total of 20 minutes. The split ratio was 10:1, the injection volume was 1 µl, the solvent delay time was 0, He was selected as the carrier gas, and the flow was adjusted to 1 ml/min. He was set to 30 ml/min, H_2 flow to 40 ml/min, and dry air flow to 400 ml/min. The external standard method was used to quantify the samples; acetic acid, butyric acid, and propionic acid standards were run in the device at different concentrations (5, 10, 12.5, 25, 50 mol/l), and their retention times in the column were determined.

RESULTS AND DISCUSSION

Initially, each medium's pH values varied between 6.70-6.98. After the incubation, no pH below these values was observed in any culture. The samples were compared with control cultures to determine the pH decrease due to short-chain fatty acids that may occur due to *E. coli* activity during the incubation process. When the control cultures (without polysaccharides) were compared with the cultures containing different concentrations of polysaccharides at the end of the incubation, it was observed that the lowest pH values were generally in cultures containing 5 mg/ml xanthan gum and 10 mg/ml pectin (except 10 mg/ml for *E. coli* F). Gel formation was observed in the media prepared at these concentrations, and problems were experienced during the pipetting stages. For these reasons, 2 mg/ml xanthan gum and 5 mg/ml pectin concentrations were selected to test the production of short-chain fatty acids.

The retention times determined for these standards are as follows: Acetic acid; 3.2 min, propionic acid; 4.1 min, butyric acid; 5.5 min. These short-chain fatty acids were not detected in any of the examined samples. Noise peaks were observed in the GC-MS chromatograms of the samples; a sample chromatogram is shown in Figure 1.

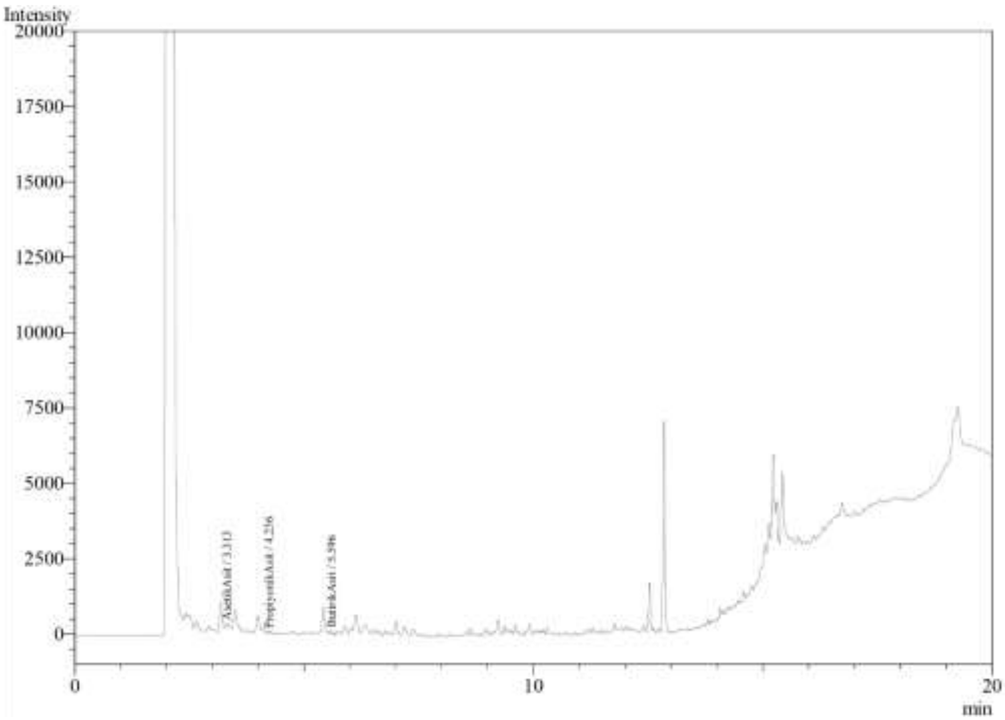


Figure 1. GC-MS chromatogram

CONCLUSIONS

Although there is no definitive information in the literature that *E. coli* can break down pectin or xanthan gum, it has been found that D-galacturonic acid units, which are monomers of pectic components, are broken down by *E. coli* in a unique way (Richard and Hilditch, 2009), and some previous studies have shown that some bacteria members of the Enterobacteriaceae family can break down pectin with their own enzymes (Abbott and Boraston, 2008). The fact that *E. coli* is an important member of the human intestinal microbiota and has strains that can be both pathogenic and probiotic makes this bacterium significant. Therefore, although the existing *E. coli* strains were tested for short-chain fatty acid production in the first step, our aim is to identify new bacterial strains that can produce short-chain fatty acids in the human intestine. In future studies, it is aimed to isolate bacteria that can use these polysaccharides and test the production of short-chain fatty acids in these strains by culturing them in media where pectin or xanthan gum is used as the sole carbon source from human feces solution.

FUNDING

This study was supported by Bayburt University Scientific Research Project Coordination with the project number 2019/01-69001-10.

REFERENCES

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. 2014 . The placenta harbors a unique microbiome. *Science translational medicine*, *6*(237), 237ra65.

Abbott, D. W. & Boraston, A. B. 2008. Structural Biology of Pectin Degradation by Enterobacteriaceae. Microbiol. Mol. Biol. Rev. 72, 301–316.

Bourquin, L. D., Titgemeyer, E. C. & Fahey, G. C. 1996. Fermentation of various dietary fiber sources by human fecal bacteria. Nutr. Res. 16, 1119–1131.

Chambers, E. S., Preston, T., Frost, G. & Morrison, D. J. 2018. Role of Gut Microbiota-Generated Short-Chain Fatty Acids in Metabolic and Cardiovascular Health. Current Nutrition Reports vol. 7 198–206.

Clemente, J. C., Ursell, L. K., Parfrey, L. W. & Knight, R. 2012. The impact of the gut microbiota on human health: An integrative view. Cell vol. 148 1258–1270.

Collins, K. H., Paul, H. A., Hart, D. A., Reimer, R. A., Smith, I. C., Rios, J. L., ... & Herzog, W. (2016). A high-fat high-sucrose diet rapidly alters muscle integrity, inflammation and gut microbiota in male rats. Scientific reports, 6(1), 37278.

Fåk, F., Jakobsdottir, G., Kulcinskaja, E., Marungruang, N., Matziouridou, C., Nilsson, U., ... & Nyman, M. 2015. The physico-chemical properties of dietary fibre determine metabolic responses, short-chain fatty acid profiles and gut microbiota composition in rats fed low-and high-fat diets. PloS one, 10(5), e0127252.

Fusco W, Lorenzo MB, Cintoni M, Porcari S, Rinninella E, Kaitsas F, Lener E, Mele MC, Gasbarrini A, Collado MC, Cammarota G, Ianiro G. 2023. Short-Chain Fatty-Acid-Producing Bacteria: Key Components of the Human Gut Microbiota. Nutrients. 6;15(9):2211.

Gentile, C. L. & Weir, T. L. 2018. The gut microbiota at the intersection of diet and human health. Science vol. 362 776–780.

Giavasis, I. 2013. Production of microbial polysaccharides for use in food. In Microbial production of food ingredients, enzymes and nutraceuticals (pp. 413-468). Woodhead Publishing.

Guillon, F., Champ, M., Thibault, J. F., & Saulnier, L. 2011. Dietary fibre functional products. In Functional foods (pp. 582-622). Woodhead Publishing.

Gulfi, M., Arrigoni, E. & Amadò, R. 2007. In vitro fermentability of a pectin fraction rich in hairy regions. Carbohydr. Polym. 67, 410–416.

Hamaker, B. R. & Tuncil, Y. E. 2014. A perspective on the complexity of dietary fiber structures and their potential effect on the gut microbiota. Journal of Molecular Biology vol. 426 3838–3850.

Holscher, H. D. 2017. Dietary fiber and prebiotics and the gastrointestinal microbiota. Gut Microbes vol. 8 172–184.

Koppel, N. & Balskus, E. P. 2016. Exploring and Understanding the Biochemical Diversity of the Human Microbiota. Cell Chemical Biology vol. 23 18–30.

Mastrocola, R., Ferrocino, I., Liberto, E., Chiazza, F., Cento, A. S., Collotta, D., ... & Collino, M. 2018. Fructose liquid and solid formulations differently affect gut integrity, microbiota composition and related liver toxicity: a comparative in vivo study. *The Journal of Nutritional Biochemistry*, *55*, 185-199.

Richard, P. & Hilditch, S. 2009. D-Galacturonic acid catabolism in microorganisms and its biotechnological relevance. Applied Microbiology and Biotechnology vol. 82 597–604.

Rosalam, S. & England, R. 2006. Review of xanthan gum production from unmodified starches by Xanthomonas comprestris sp. Enzyme Microb. Technol. 39, 197–207.

Schnizlein, M. K., Vendrov, K. C., Edwards, S. J., Martens, E. C., & Young, V. B. 2020. Dietary xanthan gum alters antibiotic efficacy against the murine gut microbiota and attenuates Clostridioides difficile colonization. Msphere, 5(1), 10-1128.

Schneider, S. M., Girard-Pipau, F., Anty, R., van der Linde, E. G., Philipsen-Geerling, B. J., Knol, J., ... & Hébuterne, X. 2006. Effects of total enteral nutrition supplemented with a multi-fibre mix on faecal short-chain fatty acids and microbiota. *Clinical Nutrition*, *25*(1), 82-90.

Schnorr, S. L., Candela, M., Rampelli, S., Centanni, M., Consolandi, C., Basaglia, G., ... & Crittenden, A. N. 2014. Gut microbiome of the Hadza hunter-gatherers. *Nature communications*, *5*(1), 3654.

Thursby, E. & Juge, N. 2017. Introduction to the human gut microbiota. Biochemical Journal vol. 474 1823–1836.

Vandamme, T. F., Lenourry, A., Charrueau, C. & Chaumeil, J. C. 2002. The use of polysaccharides to target drugs to the colon. Carbohydrate Polymers vol. 48 219–231.

Wicker, L., Kim, Y., Kim, M. J., Thirkield, B., Lin, Z., & Jung, J. 2014. Pectin as a bioactive polysaccharide–Extracting tailored function from less. Food Hydrocolloids, 42, 251-259.

Willats, W. G. T., Knox, J. P. & Mikkelsen, J. D. 2006. Pectin: New insights into an old polymer are starting to gel. Trends Food Sci. Technol. 17, 97–104.

DETERMINATION OF FATTY ACID COMPOSITION CHANGES IN SOME EDIBLE SEED WATERMELON GENOTYPES

*Seher TOPRAK, Ömer Faruk COŞKUN**

[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Hatay, Türkiye*

**Corresponding author's e-mail: omerfaruk.coskun@mku.edu.tr*

ABSTRACT

The increase in food consumption has revealed the necessity of making the best use of existing food resources. Some genotypes of watermelon, one of the important vegetable species, have snack potential due to their seed characteristics. This study was carried out to determine the seed oil ratio and fatty acid composition values of some edible seeded watermelon genotypes. Oil was obtained from the seeds of twenty-four watermelon genotypes. The oil ratio and composition of the oils obtained were determined. According to the results obtained, it was determined that watermelon seeds contained an average of 15.93% oil (on a dry basis), and this oil contained 59.75% linoleic acid, 20.63% oleic acid, 11.23% palmitic acid, and 7.53% stearic acid. The highest linoleic acid content was determined in genotype number 1, and the highest palmitic acid content was determined in genotype number 11. The results of this study can be used in breeding strategies to develop edible seeded watermelon varieties.

Keywords: *Citrullus lanatus*, Fatty Acid Composition, seed

INTRODUCTION

Plants belonging to the cucurbitaceae family have various health-promoting properties such as anti-inflammatory, antihistamine, muscle relaxant and antiulcer properties. With the increasing awareness of their health benefits, interest in cucurbitaceae is increasing (Akuamoah et al., 2018; Armesto et al., 2020). Watermelon (*Citrullus lanatus*) is a juicy and sweet-flavored vegetable species belonging to the Cucurbitaceae family, which includes 130 genera and 800 species, and is intensively cultivated worldwide. In 2022, global watermelon production exceeded 99.9 million tons on an area of 2.92 million hectares (FAO, 2024). Fruit traits in watermelon genetic resources are highly variable depending on the genotype and climatic conditions. Commonly cultivated watermelon varieties have fewer seeds and fibrous flesh compared to wild species.

The waste produced by agricultural industries worldwide exceeds 2 billion tons (Barbi et al., 2020). According to FAO, these by-products are food losses that cause a decline in the consecutive food production supply chain. There is a worldwide concern about agricultural industrial residues that cause both environmental pollution and economic losses. These losses can be overcome by implementing the environmental economics model that will help manage waste use. After the watermelon fruit is consumed, large amounts of watermelon peel and seed waste occur.

Although watermelon seeds are generally considered a waste product, more and more researchers are showing great interest in using and emphasizing their nutritional value and phytochemical profiles. Watermelon seeds can be consumed as a snack in some countries and regions. In addition to being consumed as a snack, watermelon seeds are also evaluated in the cosmetics, pharmaceutical and food industries. Since watermelon seeds contain sufficient amounts of unsaturated fatty acids, linoleic acid and oleic acid, they provide a cure for various diseases (Sodeke, 2005). In addition, watermelon seed oil is a good source of essential fatty acids and other phenolic substances in varying amounts depending on the watermelon variety (Ouassor et al., 2020). The most abundant fatty acid in watermelon seed oil is linoleic acid. Watermelon seed oil has anti-inflammatory and antimicrobial properties (Eidangbe et al., 2010; Madhavi et al., 2012). In addition, watermelon seeds are rich in Ca, Mg, Fe, and Zn minerals (Sabahelkhier et al., 2011; Garba et al., 2014). Morphological and molecular characterization studies of different watermelon genotypes have been carried out (Toprak et al., 2023; Coskun et al., 2024a, b). However, studies on the fatty acid content of watermelon genotypes with high snack potential are not sufficient. There may also be significant variations in seed fatty acid profile among watermelon genotypes. Therefore, it is important to determine the oil content of watermelon genotypes with high snack potential. This study aims to determine the oil content and fatty acid composition of seeds belonging to different watermelon genotypes.

MATERIALS AND METHODS

In this study, 24 watermelon genotypes with high snack watermelon potential were used. Samples were stored at +4°C for 12 months. Oils were extracted from seed samples of all genotypes simultaneously. Nine grams of dried seed samples from all genotypes were used for oil extraction and a Soxhlet apparatus was used. Hexane (Merck KGaA, Darmstadt, Germany) was used as the solvent. The esterification of fatty acids was done according to the method of David et al. (2003). Fatty acids were analyzed by a GC. Chromatographic separation was carried out using a (30 m × 0.25 mm ID, 0.25 μm film thickness DB-Wax) column equipped with a flame ionization detector (FID). Results were expressed as a % value.

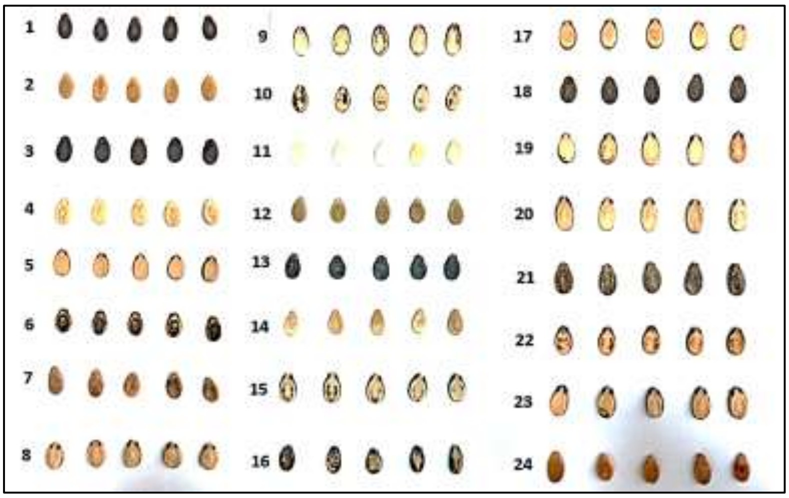


Figure 1. Seeds of different watermelon genotypes

RESULTS AND DISCUSSION

In our study, fatty acid profiles of 24 watermelon seed samples with high snack potential were examined. Saturated and unsaturated fatty acids were determined by GC analysis. In this study, palmitic acid, stearic acid, oleic acid, linoleic acid, arachidic acid and docosahexaenoic acid were measured in watermelon genotypes (Table 1).

Table 1. Fatty acid composition in watermelon genotypes

Genotyp	Oil Content (%)	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1n9)	Linoleic acid (C18:2n6c)	Arachidic acid (C20:0)	Docosahexaenoic acid (C22:6n3)
1	15.59	13.07	5.34	15.08	66.51	0.01	0.01
2	16.63	12.99	9.33	16.45	60.39	0.44	0.40
3	19.30	10.19	8.64	15.48	64.76	0.30	0.62
4	17.14	10.27	6.67	20.41	61.69	0.25	0.70
5	14.10	11.92	9.27	17.35	60.65	0.43	0.39
6	14.09	11.67	6.06	21.40	60.20	0.32	0.35
7	14.98	11.74	10.63	21.36	55.40	0.42	0.45
8	13.42	10.59	7.74	17.24	63.43	0.29	0.70
9	13.87	10.48	6.26	16.16	66.16	0.25	0.69
10	20.42	10.26	8.68	22.84	57.25	0.36	0.61
11	9.99	13.69	7.03	32.11	46.42	0.45	0.31
12	16.28	11.60	7.18	19.47	61.01	0.31	0.44
13	12.86	11.28	6.07	19.63	62.20	0.30	0.51
14	10.28	11.81	8.31	38.53	40.16	0.85	0.34
15	17.21	11.29	6.71	19.11	62.11	0.31	0.47
16	20.54	10.02	6.77	16.01	66.20	0.26	0.74
17	18.43	10.51	8.45	26.15	53.96	0.30	0.63
18	15.35	11.20	6.40	21.36	60.36	0.32	0.36
19	18.07	11.26	7.97	20.22	59.52	0.30	0.75
20	19.73	9.80	7.10	16.79	65.43	0.24	0.64
21	12.50	10.08	7.26	19.88	61.79	0.35	0.64
22	19.97	11.65	6.32	22.71	58.53	0.30	0.50
23	16.24	10.43	6.53	20.97	61.06	0.31	0.71
24	15.27	11.67	10.12	18.29	58.83	0.43	0.65
Mean	15.93	11.23	7.53	20.63	59.75	0.34	0.53

Seed oil content varied between 9.9% and 20.54% among genotypes and the average was 15.93%. The highest oil content was determined in genotype 16 and the lowest oil content was determined in genotype 11. The first five genotypes with the highest oil content are genotypes 16 (%20.54), 10 (%20.42), 22 (%19.97), 20 (%19.73) and 3 (%19.30), respectively. In previous studies, the oil content of watermelon seeds was between 10-35% and this ratio may vary depending on the genotype (Ziyada and Elhussien, 2008; Mahla et al., 2018). In this study, the oil content was determined between 9.99-20.54% among genotypes and is consistent with the literature. The total fat content value in this study is lower than the total fat content value determined by Zarifikhosroshahi and Ergun (2021). This may be due to the difference between genotypes.

In terms of fatty acid content, 6 different fatty acids were determined in watermelon genotypes. The fatty acid with the highest content was linoleic acid. Then, oleic acid, palmitic acid, stearic acid, docosahexaenoic acid and arachidic acid were determined, respectively. In a study, it was determined that watermelon seed oil contained 59.6% linoleic acid (18:2n-6) and 78.4% total unsaturated fatty acids. The dominant fatty acid in the oil was linoleic acid, followed by oleic, palmitic and stearic acids (El-Adaway and Taha, 2001a,b). These results are similar to our findings. In his study, Logaraj (2010) similarly determined the highest linoleic acid, oleic acid and palmitic acid in watermelon seeds. The main composition of the seed oil is unsaturated fatty acids, including linoleic acid, which helps lower cholesterol and high blood pressure levels in humans.

Linoleic acid values were found to be 59.75% on average in all genotypes. The highest value was measured in genotype number 1 (66.51%) and the lowest value was measured in genotype number 14 (40.16%). These values are similar to previous studies (Logaraj, 2010; El-Adaway and Taha, 2001a). The linoleic acid values determined in our study are higher than the values determined by Zarifikhosroshahi and Ergun (2021). The reason for the differences may be the difference in the material investigated. Oleic acid values were found to be 20.63% on average in all genotypes. The highest value was measured in genotype number 14 (38.53%), and the lowest value was measured in genotype number 1 (15.08%). Oleic acid values were found to be higher than some previous studies (Logaraj, 2010; Zarifikhosroshahi and Ergun, 2021). Palmitic acid values were found to be 11.23% on average in all genotypes. The highest value was measured in genotype number 11 (13.69%), and the lowest value was measured in genotype number 20 (9.80%). The palmitic acid values determined in our study are lower than the values determined by Zarifikhosroshahi and Ergun (2021). The reason for the differences may be the difference in the investigated material.

Stearic acid values were found to be 7.53% on average in all genotypes. The highest value was measured in genotype number 7 (10.63%), and the lowest value was measured in genotype number 1 (5.34%). The stearic acid values determined in our study are lower than the values determined by Zarifikhosroshahi and Ergun (2021). The reason for the differences may be the difference in the investigated material. Docosahexaenoic acid values were found to be 0.53% on average in all genotypes. The highest value was measured in genotype number 19 (0.75%), and the lowest value was measured in genotype number 1 (0.01%). Arachidic acid values were found to be 0.34% on average in all genotypes. The highest value was measured in genotype number 14 (0.85%), and the lowest value was measured in genotype number 1 (0.01%).

The development of sustainable economic systems and the evaluation of waste products through the adoption of an environmental economics approach in agricultural enterprises have been the main priorities of the scientific community in recent years. Fatty acids are used as the basic building blocks of biological membranes, long-term energy storage, and precursors of hormones. The human body cannot synthesize omega-3 and omega-6 fatty acids, which are called essential fatty acids, and must be obtained through diet. The results of this study have proven that seeds of watermelon genotypes with high snack potential are a good source of high omega-6 fatty acids. Therefore, seed oil can be considered a well-known and healthy source of human nutrition. Breeding studies can be continued by taking into account the fatty acid composition data within the examined genotypes and thus contributing to a variety of development studies.

REFERENCES

Akuamoah, F., Odamtten, G.T., Kortei, N.K. 2018. Influence of gamma irradiation on the colour parameters of dry smoked shrimps (*Penaeus notialis*). Food Res., 2, 350–355.

Armesto, J., Rocchetti, G., Senizza, B., Pateiro, M., Barba, F.J., Domínguez, R., Lorenzo, J.M. 2020. Nutritional characterization of Butternut squash (*Cucurbita moschata* D.): Effect of variety (Ariel vs. Pluto) and farming type (conventional vs. organic). Food Res. Int., 132, 109052.]

Barbi, R.C.T., Hornung, P.S., Avila, S., Alves, F.E.D.S.B., Beta, T., Ribani, R.H. 2020. Ripe and unripe inaja (*Maximilia maripa*) fruit: A new high source of added value bioactive compounds. Food Chem. 2020, 331, 127333.

Biswas, R. 2017. A comprehensive review on watermelon seed oil – An underutilized product. IOSR J Pharm 7(11): 01–07.

Coşkun, Ö. F., Toprak, S., Mavi, K. 2024a. Determination of genetic diversity of edible-seeded watermelon genotypes using SRAP markers. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 29(1), 29-37.

Coşkun, Ö.F., Toprak, S., Mavi, K. 2024b.Some seed properties and molecular analysis with inter-primary binding site (iPBS) retrotranposons markers of edible-seeded watermelon genotypes. Genet Resour Crop Evol 71, 3151–3162.

David, F., Sandra, P., Wylie, P. 2003. Improving the analysis of fatty acid methyl esters using retention time locked method and retention time databases," Application Note 5990-4822EN, Agilent Technologies publication 5988-5871EN.

Eidangbe, G.O., Ojieh, G., Idonije, B., Oluba, O. 2010. Palm oil and Egusi melon oil lower serum and liver lipid profile and improve antioxidant activity in rats fed a high fat diet. J Food Technol 8: 154–158.

El-Adaway, T.A., Taha, K. 2001a. Characteristics and composition of different seed oils and flours. Food Chem., 74:47–54.

El-Adaway, T.A., Taha, K. 2001b. Characteristics and composition of watermelon, pumpkin, and paprika seed oils and flours. J Agric Food Chem., 49:1253–9.

FAOSTAT 2024. Statistical database. Food and agriculture organization of the United Nations. Available from http://www.fao.org/faostat/en/#data/QC

Garba, Z.N., Galadima, A., Siaka, A.A. 2014. Mineral composition, physicochemical properties and fatty acids profile of *Citrullus vulgaris* seed oil. Res J Chem Sci, 4 (6): 54-57.

Logaraj, T.V. 2010. Studies on selected plants and microbes with special reference to polyunsaturated fatty acids. PhD Thesis, University of Mysore, Karnataka, India.

Madhavi, P., Rao, M., Vakati, K., Rahman, H., Eswaraiah, M.C. 2012. Evaluation of anti-inflammatory activity of *Citrullus lanatus* seed oil by in-vivo and in-vitro models. Int Res J Pharm App Sci 2(4): 104–108.

Mahla, H.R., Rathore, S.S., Venkatesan, K., Sharma, R. 2018. Analysis of fatty acid methyl esters and oxidative stability of seed purpose watermelon (*Citrullus lanatus*) genotypes for edible oil. J Food Sci Technol 55(4): 1552–1561.

Ouassor, I., Aqil, Y., Belmaghraoui, W., Hajjaji, S.E. 2020. Characterization of two Moroccan watermelon seeds oil varieties by three different extraction methods. OCL 27: 13.

Sabahelkhier, M.K., Ishag, K.E.A., Sabir, A.K. 2011. Fatty acid profile, ash composition and oil characteristics of seeds of watermelon grown in Sudan. Brit J Sci, 1 (2): 76-80

Sodeke, V.A. 2005. Extraction of oil from water melon seed and analysis. Quarterly Res Service, 25-30.

Toprak, S., Coşkun, Ö. F., Mavi, K. 2023. Çerezlik karpuz genotiplerinin ISSR tekniği ile moleküler karakterizasyonu. Erciyes Tarım Ve Hayvan Bilimleri Dergisi, 6(1), 51-58.

Zarifikhosroshahi, M., Ergun, Z. 2021. The effect of storage temperature on the composition of fatty acids in Crimson Sweet (*Citrullus lanatus* var. l*anatus*) watermelon Cultivar seeds. Journal of the Institute of Science and Technology, 11(2): 839-845.

Ziyada, A.K., Elhussien, S.A. 2008. Physical and chemical characteristics of *Citrullus lanatus* var. *colocynthoide* seed oil. J Phys Sci 19(2): 69–75.

EFFECT OF GIBBERELLIC ACID ON WATERLOGGING STRESS IN MELON

*Seher TOPRAK, Ömer Faruk COŞKUN**
[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Horticulture, Hatay, Türkiye*
**Corresponding author's e-mail: omerfaruk.coskun@mku.edu.tr*

ABSTRACT

Melon (*Cucumis melo* L.) is an economically important summer vegetable species with an aromatic taste and high nutritional value, produced in large quantities worldwide. Waterlogging has become one of the most common stress factors threatening crop production and food security worldwide. Waterlogging seriously impairs the growth and productivity of agricultural products. In this study, we aimed to determine the effect of waterlogging stress on melon and whether gibberellic acid (GA_3) can change stress conditions. Waterlogging stress conditions caused significant changes in almost all measured parameters in melon. Only PAR (photosynthetic active radiation) values did not change statistically. It was determined that stress tolerance could be increased in some parameters in the group where GA_3 was applied together with waterlogging stress. SPAD (leaf chlorophyll content) values were defined as 30.36±0.89 in the control group, 23.55±1.69 in the waterlogging group, and 22.65±174 in the GA_3+waterlogging group. It was determined that the GA_3 application reduced visual damage under waterlogging stress conditions. In this study, it was determined that this flooding stress significantly negatively affected the melon. Although GA_3 application could improve some parameters in melon under waterlogging stress, its healing effect on many parameters was limited.

Keywords: giberellic acid; melon; tolerance; waterlogging stress

INTRODUCTION

Plants face various abiotic stress factors such as heavy metals, high temperature and waterlogging throughout their life cycle, which cause significant yield losses (Zhou et al., 2020; Sun et al., 2020). Environmental stress affects plant growth and development (Li et al., 2021; Coskun, 2023). Climate change has caused an increase in extreme weather events (Walter et al., 2016). Global warming and the increasing frequency of extreme weather events cause waterlogging stress to become more severe (Chen, 2018).

Floods affect more than 10% of global arable land yearly (Jackson and Colmer, 2005). Waterlogging stress can occur due to excessive rainfall events, poor soil drainage, sensitive genotype, excessive irrigation or a combination of these (Hidalgo et al 2010; Liu et al., 2020). Excess water in the soil causes saturation of soil pores, reduced oxygen availability and impaired gas exchange in tissues. In response to plant water deficit, changes in root anatomy and architecture (Morris et al., 2017). Excess water in the soil reduces the oxygen in the root environment. This causes hypoxia for the roots (Sasidharan et al., 2007). Long-term anaerobic respiration can cause the accumulation of harmful substances in the rhizosphere. Hypoxia conditions in plants negatively affect plant growth and yield (Salvatierra et al., 2020). Hypoxia can cause a decrease in net photosynthesis in plants (Habibi et al., 2023) and deterioration in water and mineral uptake (Xie et al., 2021). Root hypoxia stress can cause leaf chlorosis, curling, leaf shedding, edge browning, necrosis, yellowing, root rot, wilting and fruit drop (McGee et al., 2021). If waterlogging stress is temporary, plants may recover. However, prolonged waterlogging results in plant death.

There are many strategies to reduce the harmful effects of waterlogging (Ahmed et al., 2013). Soil tillage and drainage systems are used to remove excess water from the soil (Manik et al., 2019). The use of raised beds may also be beneficial (Sayre et al., 2004). In addition to soil management, nutrient application and genetic manipulations can be used to combat waterlogging stress. In the past, little attention has been paid to fertilizer application to alleviate waterlogging. Post-waterlogging plant fertilization can be a practical and cost-effective method to alleviate waterlogging damage (Chae et al., 2018). Plants have evolved complex mechanisms involving gene expression and regulatory networks to mitigate the effects of stress factors (Sun at al., 2020). An important component of the ability of plants to withstand waterlogging is the formation of adventitious roots (Zhang et al., 2017).

Plant species and varieties may respond differently to waterlogging stress. Some varieties can tolerate water stress for a long time. Plants with high tolerance to waterlogging stress can accumulate more nutrients in their roots during stress periods and produce more aerobic tissue. Plant hormones have an important effect on both developmental processes and stress response throughout the lifespan of plants. Previous studies have reported that ethylene, auxin, gibberellin, abscisic acid, salicylic acid, and jasmonic acid are involved in responses to waterlogging stress and mediate many pathways necessary for waterlogging tolerance (Vidoz et al., 2010; Zhang et al., 2021). Although significant progress has been made in the development of waterlogging mitigation practices, the effectiveness of GA_3 application is not yet sufficiently known.

Melon (*Cucumis melo* L.) is an economically important summer vegetable with an estimated production of over 42 million tons worldwide in 2020 according to the Food and Agriculture Organization of the United Nations (FAO). Waterlogging stress can disrupt the normal growth and development of melon and even lead to plant death. Waterlogging stress seriously affects the quality and yield of melon in rainy regions (Zhang et al., 2021). This study aimed to determine the effect of waterlogging on melon plants and the curative effect of GA_3 application.

MATERIALS AND METHODS

The experiment was carried out in Hatay Mustafa Kemal University Research Greenhouse between April 2024 and June 2024. One hybrid melon variety was used as material in the experiment. Healthy and smooth plants were selected for waterlogging stress application. The pots were placed in plastic containers filled with tap water up to the top of the hypocotyls. Waterlogging stress application was continued for ten days and waterlogging depth was kept constant throughout the experiment. Control plants without waterlogging were watered normally in plastic containers. In the other group, GA_3 (50 mg L -1) was applied together with waterlogging stress. For morphological and physiological analysis, leaves were collected after ten days of waterlogging stress and leaves of control plants were sampled at the same time. Two biological replicates were used, 10 seedlings in each replicate. Statistical analysis of experimental data was performed using SPSS. Tukey's HSD test was used to determine the significant difference between the means ($p < 0.01$). The mean ± standard error was used to present to explain statistical analysis results.

RESULTS AND DISCUSSION

The effect of waterlogging stress on some parameters in melon was determined. It was also determined whether GA_3 application had a healing effect on plants under waterlogging stress. For this purpose, root length, plant length, fresh weight, dry weight, stem diameter, leaf number, visual damage, leaf chlorophyll content (SPAD) and photosynthetic active radiation (PAR) measurements were made.

Table 1. Root length, plant length, fresh weight and dry weight values

		Mean±Std. Error	**Minimum**	**Maximum**
Root Length (cm)	**Control**	50.00±8.09a	29.00	75.00
	Waterlogging	21.16±0.98b	17.00	24.00
	GA_3+Waterlogging	20.00±1.84b	14.00	27.00
	Mean	29.23±4.06	14.00	75.00
Plant Length (cm)	**Control**	111.80±9.75a	92.00	136.00
	Waterlogging	63.16±3.60b	56.00	80.00
	GA_3+Waterlogging	64.83±4.86b	44.00	77.00
	Mean	78.05±6.38	44.00	136.00
Fresh Weight (g)	**Control**	24.40±1.49a	20.10	28.90
	Waterlogging	11.12±1.27b	7.61	16.62
	GA_3+Waterlogging	10.46±0.57b	8.82	12.42
	Mean	14.79±1.66	7.61	28.90
Dry Weight (g)	**Control**	2.48±0.16a	1.85	2.76
	Waterlogging	1.16±0.15b	0.84	1.80
	GA_3+Waterlogging	1.06±0.05b	0.94	1.27
	Mean	1.51±0.17	0.84	2.76

Root length was determined as 29.23±4.06 cm on average, plant length as 78.05±6.38 cm on average, fresh weight as 14.79±1.66 g on average and dry weight as 1.51±0.17 g on average (Table 1). Root length was determined as 50.00±8.09 cm in control plants. Root length decreased as a result of waterlogging stress, and GA_3 application did not have a significant positive effect. Plant length was determined as 111.80±9.75 cm in control plants. Plant length decreased as a result of waterlogging stress, and GA_3 application did not have a significant curative effect. Fresh weight was determined as 24.40±1.49 g in control plants. Fresh weight decreased as a result of waterlogging stress, and GA_3 application did not have a significant positive effect. Dry weight was determined as 2.48±0.16 g in control plants. Dry weight decreased as a result of waterlogging stress, and GA_3 application did not have a significant positive effect. Waterlogging stress had negative effects on root length, plant length, fresh weight and dry weight values. No statistically significant effect of GA_3 application was determined on these parameters.

Table 2. Stem diameter, leaf number values and visual damage

		Mean±Std. Error	Minimum	Maximum
Stem Diameter (mm)	**Control**	4.75±0.24c	4.27	5.45
	Waterlogging	4.94±0.12b	4.60	5.42
	GA_3+Waterlogging	5.42±0.24a	4.55	6.21
	Mean	5.05±0.13	4.27	6.21
Number of Leaves	**Control**	12.60±0.40a	12.00	14.00
	Waterlogging	8.00±0.44b	7.00	10.00
	GA_3+Waterlogging	8.00±0.25b	7.00	9.00
	Mean	9.35±0.56	7.00	14.00
Visual Damage Changes	**Control**	0.00±0.00c	0.00	0.00
	Waterlogging	2.16±0.16a	2.00	3.00
	GA_3+Waterlogging	1.50±0.34b	1.00	3.00
	Mean	1.29±0.25	0.00	3.00

The average stem diameter was determined as 5.05±0.13 mm, the average leaf number was determined as 9.35±0.56 and the average visual damage was determined as 1.29±0.25 (Table 2). The stem diameter was determined as 4.75±0.24 mm in control plants. As a result of waterlogging stress, trunk diameter values increased, and the highest trunk diameter values were obtained as a result of GA_3 application. The number of leaves was determined as 12.60±0.40 in control plants. As a result of waterlogging stress, leaf number values decreased, and GA_3 application did not have a significant positive effect. Visual damage was not observed in control plants. As a result of waterlogging stress, visual damage increased, and it was determined that GA_3 application reduced visual damage. No positive effect of GA_3 application was determined in terms of leaf number. However, it was determined that GA_3 application had positive results in terms of visual damage and stem diameter values.

Table 3. SPAD and PAR values

		Mean±Std. Error	Minimum	Maximum
Leaf Chlorophyll Content (SPAD)	**Control**	30.36±0.89a	27.50	32.30
	Waterlogging	23.55±1.69b	17.40	29.90
	GA_3+Waterlogging	22.65±1.74b	15.90	27.70
	Mean	25.23±1.18	15.90	32.30
Photosynthetic Active Radiation (PAR)	**Control**	69.40±0.87a	66.00	71.00
	Waterlogging	69.66±0.88a	67.00	72.00
	GA_3+Waterlogging	61.33±5.53a	38.00	72.00
	Mean	66.64±2.11	38.00	72.00

SPAD was determined as 25.23±1.18% and PAR was determined as 66.64±2.11% on average (Table 3). SPAD values were determined as 30.36±0.89% in control plants. As a result of waterlogging stress, SPAD values decreased, and GA_3 application did not have a significant positive effect. PAR values were determined as 69.40±0.87% in control plants. As a result of waterlogging stress, PAR values were similar to the control group, and GA_3 application did not have a significant positive effect. Waterlogging stress had a negative effect on physiological values related to photosynthesis such as SPAD, and positive effects of GA_3 application were not determined. A total of nine parameters were measured. As a result of waterlogging stress, PAR values did not change and stem diameter values increased. Negative effects of waterlogging stress were determined in the other seven parameters. Again, no remedial effect of GA_3 application was found in the same seven parameters. An increase in stem diameter occurred as a result of GA_3 application. In addition, GA_3 application reduced the degree of visual damage.

One of the most important problems encountered in vegetable cultivation is waterlogging. Plants exposed to waterlogging respond to the damage caused by waterlogging stress by changing their growth morphology. In some soil types, waterlogging causes ion toxicity in the soil, which can affect cell permeability. Suppressed plant respiration negatively affects stomatal status, chlorophyll content, and photosynthesis rate. It has been determined that waterlogging stress causes damage to chloroplasts in plants and negatively affects photosynthesis (Zhou et al., 2023). In this study, photosynthetic parameters such as SPAD and PAR were similarly negatively affected under waterlogging stress. Waterlogging stress creates an oxygen-deficient growth environment in the plant root zone. As a result of oxygen deficiency, nutrient transport by the root system is inhibited and leaf yellowing occurs in the above-ground parts (Wang et al., 2007). In this study, visual damage increased with stress.

As a result of waterlogging stress, plant root respiration is affected (Zhi et al., 2008). This situation causes plants that want to maintain root respiration to grow more roots. In their study, Mi et al. (Mi et al., 2018) determined that adventitious roots formed in waterlogging-resistant melon plants were more numerous and longer. It has been reported that various morphological changes, including aerenchyma development, adventitious root formation, shoot elongation and leaf epinasty, can alleviate the negative effects of hypoxic environment caused by waterlogging stress (Dawood et al., 2016; Herzog et al., 2016; Ni et al., 2019)

In some previous studies, it has been determined that plant nutrient and hormone applications can improve waterlogging stress. In a study, it was determined that nitrogen application in wheat plants under waterlogging stress increased seed production (Kisaakye et al., 2017). Similarly, in different studies, fertilizer application under waterlogging stress conditions has shown positive effects (Robertson et al., 2009; Wu et al., 2014). In some studies, it has been reported in previous studies that gibberellin, abscisic acid, salicylic acid and jasmonic acid mediate many pathways required for waterlogging tolerance (Vidoz et al., 2010; Zhang et al., 2021). In their study, Islam et al. (2022) determined that GA_3 application in *Vigna radiata* plant under waterlogging stress caused improvement in many parameters. In this study, the ameliorative effect of gibberellic acid under water stress conditions was investigated. Waterlogging stress had negative effects in terms of root length, plant length, fresh weight and dry weight values. No statistically significant effect of GA_3 application was determined on these parameters. No positive effect of GA_3 application was determined in terms of leaf number.

However, it was determined that GA_3 application had positive results in terms of visual damage and stem diameter values. Waterlogging stress had a negative effect on physiological values related to photosynthesis such as SPAD, and positive effects of GA_3 application were not determined. In this study, it was determined that this waterlogging stress significantly affected melon negatively, and although GA_3 application could provide improvement in some parameters in melon under waterlogging stress, its improvement effect on many parameters was limited. More studies are needed on the mechanism of waterlogging stres tolerance, screening of waterlogging stres tolerant varieties and introduction of melon varieties.

REFERENCES

Ahmed, F., Rafii, M., Ismail, M.R., Juraimi, A.S., Rahim, H., Asfaliza, R., Latif, M.A. 2013. Waterlogging tolerance of crops: Breeding, mechanism of tolerance, molecular approaches, and future prospects. BioMed Res. Int., 2013, 963525.

Chae, H.-S., Noh, H.-J., Song, W.S., Cho, H.-H. 2018. Efficiency and effectiveness of vitamin C-substrate organo-mineral straight fertilizer in lettuce (*Lactuca sativa* L.). Chem. Biol. Technol. Agric., 5, 4.

Chen, Y.M. 2018. Physiological Research on the Response of KiwifruitTolerant-Rootstock to Hypoxia Stress; Zhejiang A&F University: Hangzhou, China.

Coskun, Ö.F. 2023. The Effect of Grafting on Morphological, Physiological and Molecular Changes Induced by Drought Stress in Cucumber. Sustainability, 15, 875. https://doi.org/10.3390/su15010875

Dawood, T., Yang, X., Visser, E.J.W., te Beek, T.A.H., Kensche, P.R., Cristescu, S.M., Lee, S., Floková, K., Nguyen, D., Mariani, C., et al. 2016. A co-opted hormonal cascade activates dormant adventitious root primordia upon flooding in Solanum dulcamara. Plant Physiol., 170, 2351–2364.

Habibi, F., Liu, T., Shahid, M.A., Schaffer, B., Sarkhosh, A. 2023. Physiological, biochemical, and molecular responses of fruit trees to root zone hypoxia. Environ. Exp. Bot., 206, 105179.

Herzog, M., Striker, G.G., Colmer, T.D., Pedersen, O. 2016. Mechanisms of waterlogging tolerance in wheat-a review of root and shoot physiology. Plant Cell Environ., 39, 1068–1086.

Hidalgo, F.D., Naidu, S., Nichter, S., Richardson, N. 2010. Economic determinants of land invasions. Rev. Econ. Stat., 92, 505–523.

Islam, M.R., Rahman, M.M., Mohi-Ud-Din, M., Akter, M., Zaman, E., Keya, S.S., Hasan, M., Hasanuzzaman, M. 2022. Cytokinin and gibberellic acid-mediated waterlogging tolerance of mungbean (*Vigna radiata* L. Wilczek). PeerJ.;10:e12862. doi: 10.7717/peerj.12862.

Jackson, M., Colmer, T. 2005. Response and adaptation by plants to flooding stress. Ann. Bot., 96, 501–505.

Kisaakye, E., Acuña, T.B., Johnson, P., Shabala, S. 2017. Improving wheat growth and nitrogen-use efficiency under waterlogged conditions. In Proceedings of the 18th Australian Agronomy Conference, Ballarat, VIC, Australia, pp. 1–4.

Li, W.H., Zeng, Y.L., Yin, F.L., Wei, R., Mao, X.F. 2021. Genome-wide identification and comprehensive analysis of the NAC transcription factor family in sunflower during salt and drought stress. Sci. Rep., 11, 19865.

Liu, K., Harrison, M.T., Ibrahim, A., Manik, S.N., Johnson, P., Tian, X., Meinke, H., Zhou, M. 2020. Genetic factors increasing barley grain yields under soil waterlogging. Food Energy Secur., 9, e238.

Manik, S., Pengilley, G., Dean, G., Field, B., Shabala, S., Zhou, M. 2019. Soil and crop management practices to minimize the impact of waterlogging on crop productivity. Front. Plant Sci., 10, 140.

McGee, T., Shahid, M.A., Beckman, T.G., Chaparro, J.X., Schaffer, B., Sarkhosh, A. 2021. Physiological and biochemical characterization of six Prunus rootstocks in response to flooding. Environ. Exp. Bot., 183, 104368.

Mi, B.B., Wu, F.F., Xie, L.L., Dai, X.Z., Zhou, H.Q. 2018. Morphological structure and physiological response of winter melon seedlings under waterlogging stress. South. J. Agric., 49, 2419–2424.

Morris, E.C., Griffiths, M., Golebiowska, A., Mairhofer, S., Burr-Hersey, J., Goh, T., von Wangenheim, D., Atkinson, B., Sturrock, C.J., Lynch, J.P., et al. 2017. Shaping 3D root system architecture. Curr. Biol., 27, R919–R930.

Ni, X.L., Gui, M.Y., Tan, L.L., Zhu, Q., Liu, W.Z., Li, C.X. 2019. Programmed cell death and aerenchyma formation in water-logged sunflower stems and its promotion by ethylene and ROS. Front. Plant Sci., 9, 1928.

Robertson, D., Zhang, H., Palta, J.A., Colmer, T., Turner, N.C. 2009. Waterlogging affects the growth, development of tillers, and yield of wheat through a severe, but transient, N deficiency. Crop Pasture Sci., 60, 578–586.

Salvatierra, A., Toro, G., Mateluna, P., Opazo, I., Ortiz, M., Pimentel, P. 2020. Keep calm and survive: Adaptation strategies to energy crisis in fruit trees under root hypoxia. Plants, 9, 1108.

Sasidharan, R., Bailey-Serres, J., Ashikari, M., Atwell, B.J., Colmer, T.D., Fagerstedt, K., Fukao, T., Geigenberger, P., Hebelstrup, K.H., Hill, R.D., et al. 2017. Community recommendations on terminology and procedures used in flooding and low oxygen stress research. New Phytol., 214, 1403–1407.

Sayre, K.D., Hobbs, P.R. 2004. The raised-bed system of cultivation for irrigated production conditions. In Sustainable Agriculture and the International Rice-Wheat System; CRC Press: Boca Raton, FL, USA, pp. 337–355.

Sun, Z., Song, Y., Chen, D., Zang, Y., Zhang, Q., Yi, Y., Qu, G. 2020. Genome-Wide Identification, Classification, Characterization, and Expression Analysis of the Wall-Associated Kinase Family during Fruit Development and under Wound Stress in Tomato (*Solanum lycopersicum* L.). Genes, 11, 1186.

Vidoz, M.L., Loreti, E., Mensuali, A., Alpi, A., Perata, P. 2010. Hormonal interplay during adventitious root formation in flooded tomato plants. Plant J., 63, 551–562.

Walter, J., Harter, D.E.V., Beierkuhnlein, C., Jentsch, A. 2016. Transgenerational effects of extreme weather: Perennial plant offspring show modified germination, growth and stoichiometry. J. Ecol., 104, 1032–1040.

Wang, P., Hu, Y.H., Wang, L.M., Liu, Q.H. 2007. Kinds of identification index and evaluation means of plant submergence endurance. North. Hortic., 11, 78–81.

Wu, J., Li, J., Wei, F., Wang, C., Zhang, Y., Sun, G. 2014. Effects of nitrogen spraying on the post-anthesis stage of winter wheat under waterlogging stress. Acta Physiol. Plant., 36, 207–216.

Xie, R., Zheng, L., Jiao, Y., Huang, X. 2021. Understanding physiological and molecular mechanisms of citrus rootstock seedlings in response to root zone hypoxia by RNA-Seq. Environ. Exp. Bot., 192, 104647.

Zhang, H., Li, G., Yan, C., Cao, N., Yang, H., Le, M., Zhu, F. 2021. Depicting the molecular responses of adventitious rooting to waterlogging in melon hypocotyls by transcriptome profiling. 3 Biotech, 11, 351.

Zhang, Q., Huber, H., Beljaars, S.J.M., Birnbaum, D., de Best, S., de Kroon, H., Visser, E.J.W. 2017. Benefits of flooding-induced aquatic adventitious roots depend on the duration of submergence: Linking plant performance to root functioning. Ann. Bot., 120, 171–180.

Zhi, L.Y., Hu, S.Z., Yu, L., You, Q. 2008. Effects of waterlogging stress on growth and leaf physiological of Euscaphis konishii Hayata seedlngs. Acta Agric. Univ. Jiangxiensis, 30, 279–282.

Zhou, R., Niu, L., Yin, J., Jiang, F., Wang, Y., Zhao, T., Zhu, W. 2023. Differences in physiological responses of two tomato genotypes to combined waterlogging and cadmium stresses. Antioxidants, 12, 1205.

Zhou, W., Chen, F., Meng, Y., Chandrasekaran, U., Luo, X., Yang, W., Shu, K. 2020. Plant waterlogging/flooding stress responses: From seed germination to maturation. Plant Physiol. Biochem., 148, 228–236.

IMMOBILIZATION OF HEAVY METAL BY CLAYS IN AGRICULTURAL SERPENTINE SOILS

Teuta Bushi[1], Aida Bani[2], Selma Mysilhaka[3], Seit Shallari[2],
[1] Department of Physics, Faculty of Forest Sciences, Agricultural University of Tirana, Albania
[2] Department of Environment and Natural Resources. Faculty of Agriculture and Environment, Agricultural University of Tirana, Tirane, Albania
[3] Departament of Biology, Faculty of Natural Sciences, University "Aleksander Xhuvani", Elbasan, Albania

Heavy metals and metalloids can accumulate in soil, with potentially toxic effects to human health and ecosystems, threatening the sustainable use and management of soil resources.
The concern of high concentration of heavy metals coming from mineralized soil is even greater when we cultivate food plants. Several procedures have been proposed to reduce the concentration of heavy metals in the soil; among them, the application of materials such as bentonite, able to absorb these elements, making them less available to plants.
The present study deals with the use of clay material as an adsorbent and Ni hyperaccumulator plant, *Odontarrhena chalcidica,* for the removal of Ni from solutions of agricultural serpentine soil in Albania, as an alternative reduce nickel availability and to reduce the risk of uptake of nickel by vegetable. *Ex-situ* experiment was conducted with serpentine soil Where we add clays and cultivated *Odontarrhena calchidica* plant. The experiment was conducted in 1 kg plastic plot. We used four doses of clays: 0.0; 10.7; 21.4 and 32.1 g kg^{-1}, corresponding to 0, 30, 60 and 90 t ha^{-1}, respectively. Experiment was with 3 replications for plots with clays and them with clays an Ni hyperaccumulator. After the 60 th day of experiment, the plants collected separating the aerial part, washing with distillated water, conditioned in paper sacks and dried in forced air stove at 65^{o} C during 48 hours. Total metal determination will conduct after digestion of soils and plants, using ICP. We measured available metals in soil of ex-situ experiment before experiment for every treatment and after 60 days. Ex situ experiment help us to know capacity of clay to immobilize metals and capacity of Ni hyperaccumulator plant to extract metal and soil remediation.

Key words: Heavy metals, hyperaccumulator plant, *Odontarrhena chalcidica*, clay, absobent

INTRODUCTION

Clay minerals have attracted much attention for metal stabilization due to their high specific area, liming (pH-increasing) effect, excellent ion exchange capacity and abundant surface hydroxyl groups[1],[2],[3], strong mechanical stability, stable chemical properties, lower cost and environmentally friendly and offer a cost-effective alternative to conventional treatment .
The concern of high concentration of heavy metals coming from mineralized soil is even greater when we cultivate food plants. Several procedures have been proposed to reduce the concentration of heavy metals in the soil; among them, the application of materials such as bentonite, able to absorb these elements, making them less available to plants.
Albanian natural clays have the ability to adsorb heavy metals and decrease their availability in the soil and the possibility of take up and accumulate in agricultural plants. The aim of this study was to evaluate the adsorption ability of natural soil components such as clays to

improve the agricultural serpentine soils. We use the clay extracted by soils collected in Prrenjas area (Domosdova field) [4],[5],[6]
The present study deals with the use of clay material as an adsorbent for the removal of Ni from solutions of agricultural serpentine soil in of Albania, as an alternative reduce nickel availability and to reduce the risk of up take of nickel by vegetable.

MATERIAL AND METHODS

We use the clay extracted by soils collected in Prrenjas area (Domosdova field) [4],[5],[6] (19°30'39" East)
Adsorption experiments carried out using metal solutions of Ni extracted from serpentine soil of Prrenjas.
We analyzed the concentrations of heavy metals before and after interaction with clay.
Experiment ex situ was conducted with hyperaccumulator (*Odontarrhena Calchidica* (Janka) Španiel & al.) plants as test plant.
The experiment was conducted in 1 kg plastic plot. We used four doses of clays: 0.0; 10.7; 21.4 and 32.1 g kg^{-1}, corresponding to 0, 30, 60 and 90 t ha^{-1}, respectively. Experiment was with 3 replications for plots with clays and them with clays an Ni hyperaccumulator.
After the 60 th day of experiment, the plants collected separating the aerial part, washing with distilled water, conditioned in paper sacks and dried in forced air stove at 65° C during 48 hours.
We measured total heavy metals in soil and plant of experiment after digestion of soils and plants by the method of atomic absorption. We measured available (Mehlich 1) metals in soil of ex-situ experiment before experiment for every treatment and after 60 days

Table1. Chemical composition of Prrenjas clay, which is used as an adsorbent in this work

Clay	SiO2%	Al2O3%	Fe2O3%	CaO%	MgO%	SO3%	rSiO2/MxOy
untreated	47.5	13.4	14.97	2.7	9.58	0.11	1.239

Ex situ Experiment with hyperaccumulator plant (*Odontarrhena Calchidica)*


PRRENJAS CLAY

RESULTS AND DISCUSSION

Ex situ experiment help us to know capacity of Clay to immobilize metals.,

Table 2. Results of chemical analysis of of agricultural soil samples from Prrenjas

Parameters	Total	Available
	mg/kg	
Cu	16.5	0.70
Fe	97920	16.37
Mg	23934	1030
Mn	1515.6	6.03
Co	127	5.28
Ni	1458.7	10.6
Ca	3578.7	644.6

Agricultural soil of Prrenjas is a typical serpentine soil. The level Mg. Fe. Ni Mn,Co are very high. The level of Ni available is 10.6mg/kg

Table 3. Results obtained for treatments of Prrenjasi soil with clay and Prrenjasi soil with clay and plants

AS= Agricultural soil

Treatments	**C0(mg/kg)**	**Ce(mg/kg)**	**q=C0-Ce(mg/kg)**	**%Ni adsorber**	**%Ni contributed by plants**
AS +C0	**10.6**				
As+Clay 10.7 g kg^{-1}		7.68	2.92	27.5	
As+Clay 21.4 g kg^{-1}		7.36	3.24	30.5	
As+Clay 32 g kg^{-1}		3.78	6.82	64.3	
As+Clay 10.7 g kg^{-1} +Plant		6.12	4.48	42.2	14.7
As+Clay 21.4 g kg^{-1} +Plant		4.48	6.12	57.7	27.2
As+Clay 32 g kg^{-1} +Plant		3.64	6.92	65.2	0.9

After the 60 th day of experiment a high % nickel was absorbed by clay and a amount of Ni was extracted by plants.

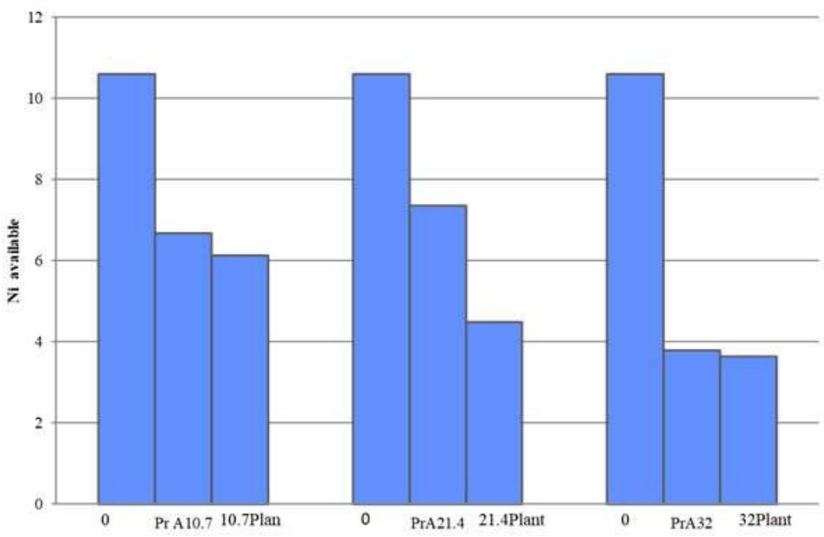


Figure 1. Ni availability in soil with different treatment

CONCLUSIONS

Result told us that clays of Prrenjas are the appropriate clay mineral for the removal of Ni. Based on the first results, the application of clays to reduce the mobility and availability of nickel is promising.

REFERENCES

[1] Doni, S., Gispert, M., Macci, C., Mattii, G. B., Manzi, D., Masini, C. M., & Masciandaro, G. Peruzzi, E., (2020). Impact of natural zeolite on chemical and biochemical properties of vineyard soils. *Soil Use and Management*. 1– 11. https://doi.org/10.1111/sum.12665

[2] Mikhail S., Zaki T. and Khalil L., J. Appl. Cat. A 227, 265-278 (2002).

[3] Sanchez, G.A., Ayuso, A.E., De Blas, J.O., Mineralogical Society Electronic Journals 34 (3), 469 (2002). U¨ lku¨ , Y., I Haluk, C¸ ., Water SA 27 (1), 15–20 (2001)

[4] Jozja, N. (2003) - "Etude de matériaux argileux albanais. Caractérisation « multiéchelle » d‟une bentonite magnésienne. Impact de l‟interaction avec le nitrate de plomb sur la perméabilité". Ph.D. Thesis, Univ. Orléans, France, 245 pp.

[5] Shallari, S., Schwartz, C., Hasko, A. & Morel, J. L. (1998). Heavy metal in soils and plants of serpentine and industrial sites of Albania. *Science of The Total Environment*, 209, 133–142. https://doi.org/10.1016/S0048-9697(98)80104-6

[6] Bani, A., Echevarria, G., Mullaj, A., Reeves, R. D, Morel, J-L., & Sulçe, S. (2009). Ni hyperaccumulation by Brassicaceae in serpentine soils of Albania and NW Greece. *Northeastern Naturalist*, 16 (Special Issue 5), 385–404. https://www.jstor.org/stable/41495828

PRELIMINARY DATA ON MACROZOOBENTHOS FROM THE SHALLOW ROCKY COAST OF HIMARA, ALBANIA.

*[1]*Brunilda Veshaj, [2]Stela Ruci, [1]Denada Sota, [1]Ina Nasto, [2]Anisa Toska,[1]Kristiana Lushnjari,[2]Sajmir Beqiraj*

[1]Department of Biology, Faculty of Technical and Natural Sciences, University "Ismail Qemali", Vlora, Albania;
[2]Department of Biology, Faculty of Natural Sciences, University of Tirana, Albania

**Corresponding author e-mail:*brunildaveshaj@gmail.com

ABSTRACT

Himara coast represents the northeastern segment of the Ionian Sea, which is situated in the southwestern part of Albania. Data from macrozoobenthic community of this area are scarce. Although studies on infralittoral and circalittoral have been more frequent, research and surveys on the shallow rocky coast of Himara have been very limited, and the few existing data on shallow macrozoobenthos have been mainly sporadic. Data represented in this paper were collected in October 2023 from 4 sampling sites along the Himara coast, in shallow water, in supralittoral and midlittoral. These are part of an ongoing long-term study that aims to know and analyze the macrozoobenthic community of this area, including species composition, abundance and quantitative characteristics of benthic invertebrate populations, their relations to algal cover and algae species, as well as other factors influencing characteristics of their populations, in a comparative way between sampling sites and sampling seasons. Preliminary data represented in this paper have shown the presence of about 130 species of benthic macroinvertebrates. At the level of large taxonomic groups, they include cnidarians, polychaetes, mollusks, crustaceans, sipunculids and echinoderms, where the predominant groups in species number and abundance were Gastropoda, Bivalvia, Amphipoda and Decapoda. Algal cover, morphological diversity of substrata, degree of coastal exposure, and environmental impact, mainly from tourism, seem to be the main factors that affect the macrozoobenthic community of this study area

Key words: Benthic invertebrates, Ionian Sea, algal cover, marine and coastal conservation.

INTRODUCTION

The rocky coast of Himara is part of the Ionian Sea and is located in the southwestern part of Albania. Benthic communities on the coast of Himara are little studied, mainly focused on macroinvertebrates, macroalgae and meadows of the seagrass Posidonia oceanica (Beqiraj et al., 2008; Beqiraj & Kashta, 2013; Beqiraj, 2014; Beqiraj & Ballesteros, 2018; Fraschetti et al., 2011; Kashta et al., 2005; Kashta et al., 2007; Kashta & Beqiraj, 2009; Pititto etal., 2009; Ruci et al., 2023.Among the four sampling sites of this study, Porto Palermo Bay has undergone the most extensive research, primarily concerning its proposed designation as a Marine Protected Area (Beqiraj et al. 2008, Beqiraj & Kashta 2013, Beqiraj 2014), proclaimed in July 2022. The other three sites, Guma, Llaman, and Qeparo, have been sparsely studied, and data availableon their benthic communities are very limited. Research on the benthic community in Himara area is crucial for understanding and assessing its biodiversity and natural values of the whole marine and coastal ecosystem in that area, its cultural and socio-economic significance, as well as for environmental conservation and management of the recently proclaimed MPA.Further, the natural resources are vital for sustainable development and directly affect the local economy of this area.

MATERIAL AND METHODS

Benthic invertebrates were collected in October 2023 from four sampling sites, Qeparo, Porto Palermo Bay, Llamani Bay, and Guma, along the Himara coast, in shallow water (Fig. 1 & Fig. 2).

Sampling was carried out according to standard methods for benthic sampling in hard bottoms (according to Bianchi et al.2003, Keklikoglou et al. 2018, Mikac et al. 2020, Salomidi 2003, Zenetos et al. 2000). Samples were taken randomly, within a standard frame of 50 cm x 50 cm, divided in 25 small squares of 10 cm x 10 cm. They were stored in formaldehyde 4% and transported to the laboratory.Taxonomic identification of benthic invertebrates was referred to Cossignani 1992; Cossignani& Ardovini 2011;Clemam checklist; D'Angello &Gargiullo 1991; Gianuzzi-Savelli, 1999, 2001, 2003; Riedl 1991; Rodríguez-Prieto et al 2015. The systematic position of invertebrates was referred to WoRMS (World Register of Marine Species).

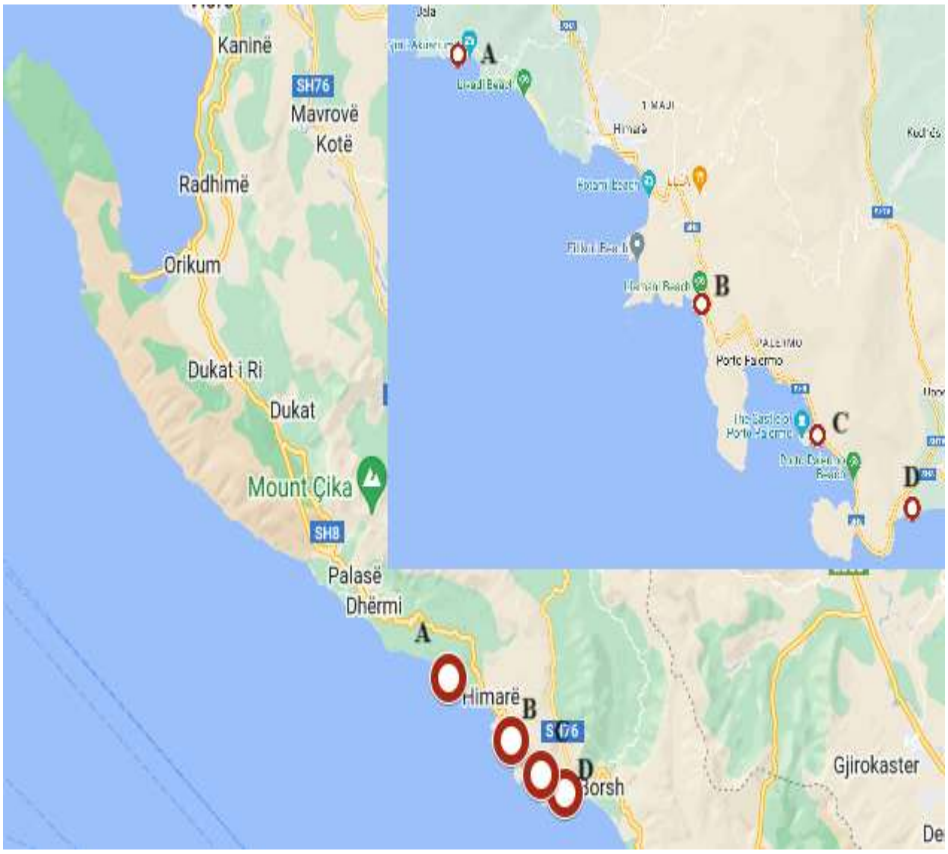


Figure 1. Map of sampling sites: a) Guma; b) Llaman;c) Porto Palermo;d) Qeparo

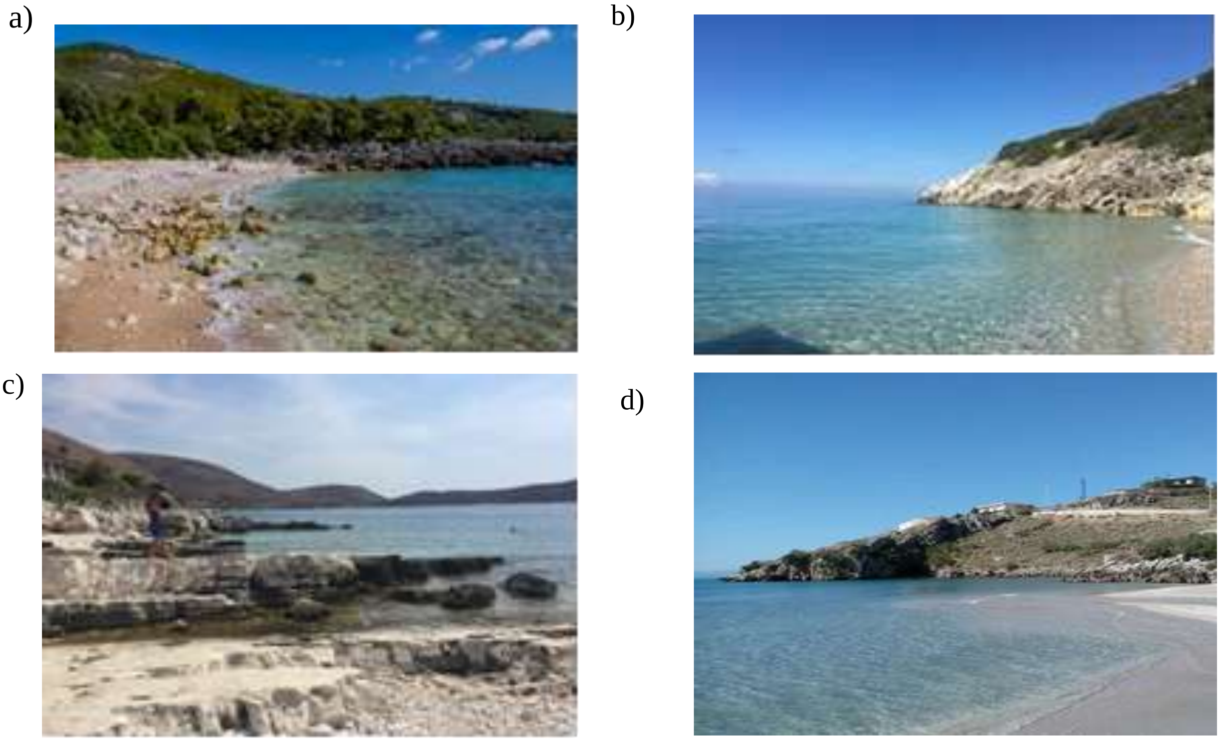


Figure 2. Photos from the sampling sites: a) Guma; b) Llaman;c) Porto Palermo; d) Qeparo

For each taxonomic group it was calculated the number of taxa at each sampling site, the abundance,and the frequency. The frequency has been calculated after the formula, $F = \frac{n}{N} x100$ (Peja 1995), which represents the proportion of the number of individuals of a species (n) in a sample to the total number of individuals (N) in that sample.

RESULTS AND DISCUSSION

130 species of benthic invertebrates were recorded in the study area. Molluscs (Gastropoda and Bivalvia) and crustaceans (Amphipoda and Decapoda) represented the highest number of taxa in the four sampling sites.

As shown in Table 1 and Figure 3, the highest number of taxa for mollusks was recorded in the Guma sampling site with 50. Crustaceans showed a high number of taxa in Qeparo 24, Porto Palermo 40, Llaman 32 and a lower number in Guma, with 18 taxa.

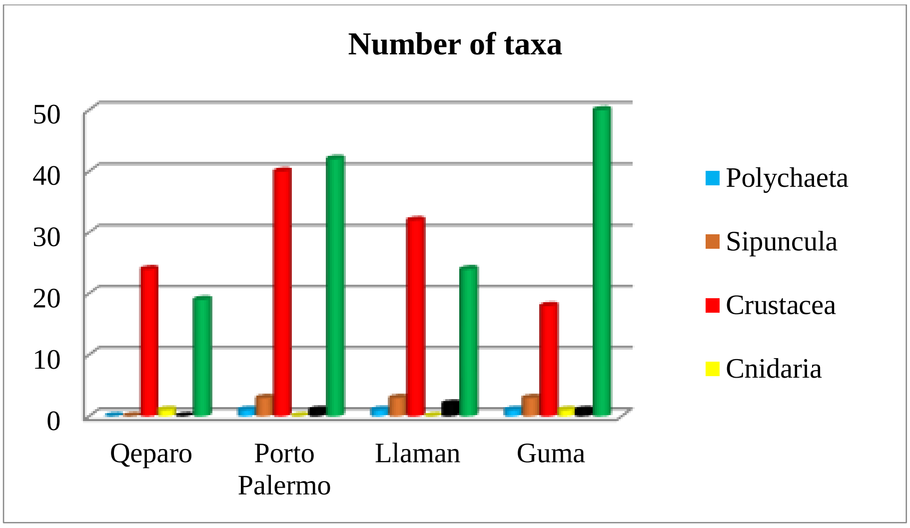


Figure 3. Number of taxa in each sampling site

The highest abundance was recorded for crustaceans (Figure 4). Molluscs were another taxonomic group that showed very large abundances in all sampling sites. Other taxonomic groups, such as Polychaeta, Sipuncula, Cnidaria and Echinodermata showed very low abundance in all sampling sites.

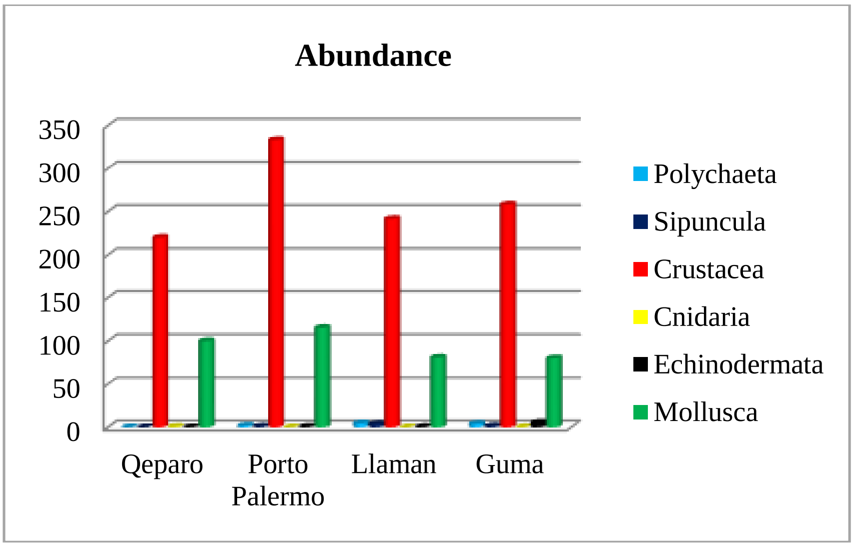


Figure 4. Abundance of benthic macroinvertebrates in each sampling site

As seen in figure 5, crustaceans have represented the highest frequency, which is almost the same in all sampling sites. For Molluscs the highest frequency was recorded in Qeparo, and it was almost the same in the other three sampling sites. It is also noted a high frequency ofCnidarians in Qeparo, compared to Porto Palermo, Llaman and Guma. The frequency of the other taxonomic groups was very low in the four sampling sites.

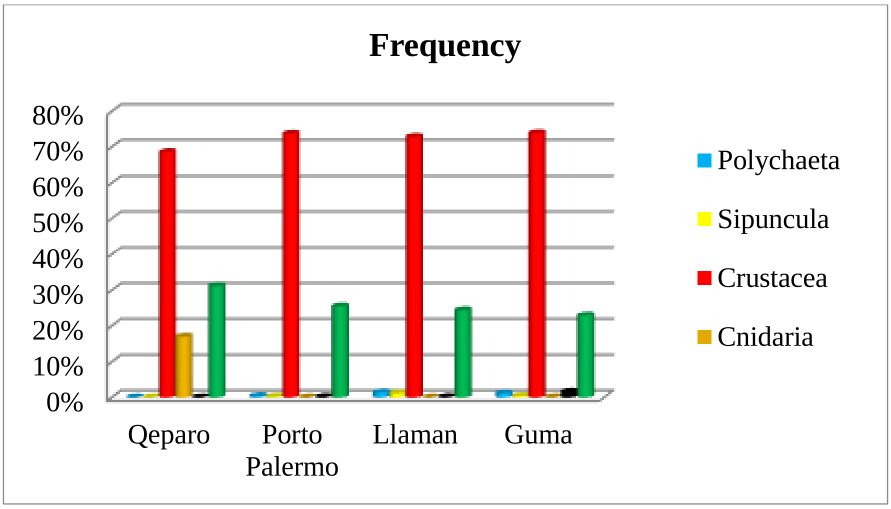


Figure 5.Frequency of benthic macroinvertebrates in each sampling site

Diversity of microhabitats, exposure degree of the coastline, macroalgal cover, and environmental impacts mainly from tourism activities, seem to be the main factors that influence the species diversity and abundance of benthic invertebrates in the Himara area.

Continuation of this study, and its integration with the assessment of water physicalchemical parameters, will give a more comprehensive understanding of the benthic community and its environmental impacts in this study area.

REFERENCES

Beqiraj, S., Kashta, L., Kuci, M., Kasemi, D., Mato. Xh, Gace. A. (2008). Benthic macrofauna of *Posidonia oceanica* meadows in the Albanian coast. Natura Montenegrina 2008/7(2): 55 –69.

Beqiraj, S., Zenetos, A. (2021). Non-indigenous species. – In “Integrated Monitoring Programme – Albania”. Eds: PAP/RAC, GEF Adriatic project. pp140 + Annexes.

Beqiraj, S., Kashta., L. (2014). Assessment and preparation of documents for the new proposed MPA Cape of Rodoni. “Improving the coverage and management effectiveness marine and coastal protected areas in Albania” (UNDP, GEF, MoE). Technical report, 61 pp.

Beqiraj, S., Ballesteros, E. (2018). Assessment of the state of marine biodiversity in the Albanian littoral. Albanian Conference on Biology and Environment (KShBM - ACBE). 28 – 30 September 2018. Prishtina.

Bianchi et al. 2003, Hard bottoms. In: Mediterranean marine benthos: a manual of methods for its sampling and study. Biologia Marina Mediterranea (2004), 11, (suppl.1): 185 – 215.

Cossignani, T. (1992). Atlante delle conchiglie Del Medio Adriatico. L’informatore PicenoEd. Ancona: 11 – 40. 17.

D’Angello, G. & Gargiullo, S. (1991). Guida alle Conchiglie Mediterranee. Fabbri S.p.A. Milano: 224 pp. 18.

Fraschetti, S., Beqiraj, S., Gačić, M., Mascle, J., Odorico, R. (2011). Scientific rationale for the proposed CIESM North Ionian Sea Marine Peace Park. CIESM Workshop Monographs. Nr. 87-96.

Giannuzzi-Savelli, R., Pusateri, F., Palmeri, A., Ebreo, C. (1999). Atlante delle conchiglie Del Mediterraneo. “Evolver” srl. Roma. Vol. 3: 7 – 18. 28.

Giannuzzi-Savelli, R., Pusateri, F., Palmeri, A., Ebreo, C. (2001). Atlante delle conchiglie Del Mediterraneo. “Evolver” srl. Roma. Vol. 7: 42 - 240. 29.

Giannuzzi-Savelli, R., Pusateri, F., Palmeri, A., Ebreo, C. (2003). Atlante delle conchiglie Del

Mediterraneo. “Evolver” srl. Roma. Vol. 4: 22 – 290.

Hazarika, L. P. (2013). Diversity indices of macroinvertebrates. Annals of Biological Research, 4 (8): 68 – 72. Kashta, L., Beqiraj, S., Mato, Xh., Xhulaj, M., Gaçe, A., Mullaj, A. (2005). The inventory of habitats with *Posidonia oceanica* and littoral habitats in Albania. Technical Report. APAWA, Tirana. 86 pp.

Kashta, L., Xhulaj, M., Mato, Xh., Beqiraj, S., Gace, A. (2007). The state of *Posidonia meadows* along the Albanian coast: general evaluation. 3-d Mediterranean Symposium on MarineVegetation. Marseilles, France: 121 – 125.

Kashta, L., Beqiraj, S. (2009). Analysis of the proposed potential Marine Protected Areas.

Millard, V. (2001). Clasification of Mollusca. Vol.2 & Vol. 3. V. Millard. South Africa: 36 – 128, 144 – 189.

Opistobranchs (Gastropoda, Heterobranchia): Shedding light on biodiversity issues. Marine Ecology, Vol. 37, Issue 6: 1239 – 1255.

Pititto, F., Grenci, S., Dedej, Z., Gace, A., Kashta, L., Beqiraj, S., Acunto, S., Bulgheri, G., Cinelli, F., Sivini, N., Greco, R., Torchia, G. (2009): Cartography and protection of the *Posidoniaoceanica* meadows along the Albanian coast. [Cartografia e protezione delle praterie di Posidonia oceanica lungo la costa albanese]. Biologia Marina Mediterranea. 16 (1): 324 –325.

Protected Areas Gap Assessment and Marine Protected Areas Development in Albania,(UNDP, GEF, MEFWA). Technical report, 81 pp.

Peja, N. 1995. Ekologjia. SHBLU. Tiranë: 249 - 253.

Ruci, S.,Kasemi, D., Nasto, I., Toska, A., Beqiraj, S. (2023). Molluscs from Himara coast, Ionian sea, Albania. V. International Agricultural, Biological & Life Science Conference,Edirne, Turkey, 18-20 September 2023: 1138- 1146.

Riedl, R. (1991). Fauna e Flora Del Mediterraneo. Franco Muzzio Editore. Padova: 105 – 472Simboura, N., & Zenetos, A. (2002). Benthic indicators to use in ecological quality classification of Mediterranean soft bottom marine ecosystems, including a neë biotic index. Mediterranean Marine Science, 3(2), 77-111.

Trainito E. Atlante di Flora e Fauna Del Mediterraneo. Il Castello srl. Milano; 2004: 10 –241.

CLEMAM, Check List of European Marine Mollusca,h://www.mnhn.fr/base/malaco.html.

Zenetos, A., Bellou, N., & Abousamra, F. (2005). The use of BENTIX in assessing ecological quality of coastal waters across the Mediterranean. INDICATORS OF STRESS IN THE MARINE BENTHOS.

Zenetos, A., Mačić, V., Jaklin, A., Lipej, L., Poursanidis, D., Cattaneo-Vietti, R., Beqiraj, S., Betti, F., Poloniato, D., Kashta, L., Katsanevakis, S., Crocetta, F. (2016). Adriatic

APPENDIX 1.

Table 2. List of taxa recorded in the four sampling sites (Qeparo, Porto Palermo, Llaman, Guma), in Himara Coast, in October 2023.

Taxa	**Qeparo**	**Porto Palermo**	**Llaman**	**Guma**
Polychaeta				
Lepidonotus clava (Montagu, 1808)		+	+	+
Sipuncula				
Aspidosiphon (Aspidosiphon) muelleri muelleri Diesing, 1851		+	+	
Golfingia (Golfingia) vulgaris vulgaris (de Blainville, 1827)		+	+	
Phascolosoma (Phascolosoma) granulatum Leuckart, 1828		+	+	
Crustacea				
Galathea sp. Fabricius, 1793	+	+	+	
Paguristes eremita (Linnaeus, 1767)		+		
Acanthonyx lunulatus (Risso, 1816)	+	+	+	+
Eriphia verrucosa (Forskål, 1775)	+	+	+	+
Inachus sp. Weber, 1795		+		
Carcinus aestuarii Nardo, 1847		+		
Pachygrapsus marmoratus (Fabricius, 1787)	+		+	
Stenothoe monoculoides (Montagu, 1813)		+		
Stenothoe marina (Spence Bate, 1857)			+	+
Liljeborgia dellavallei Stebbing, 1906	+			
Nototropis massiliensis (Bellan-Santini, 1975)				
Dexamine spinosa (Montagu, 1813)		+	+	+
Ampelisca sarsi Chevreux, 1888		+		
Caprella telarpax Mayer, 1890			+	
Caprella andreae Mayer, 1890		+		
Caprella mitis Mayer, 1890		+	+	
Caprella penantis Leach, 1814		+		
Caprella danilevskii Czerniavsky, 1868		+	+	
Caprella acanthifera Leach, 1814		+		
Caprella grandimana Mayer, 1882			+	
Caprella rapax Mayer, 1890			+	
Ampithoe ramondi Audouin, 1826	+	+	+	+
Pleonexes helleri (Karaman, 1975)	+	+	+	+
Autonoe spiniventris Della Valle, 1893				
Aora gracilis (Spence Bate, 1857)		+		
Leptocheirus pectinatus (Norman, 1869)		+		
Corophium sp Latreille, 1806		+		
Cymadusa filosa Savigny, 1816			+	
Neogammarus adriaticus Karaman, 1973	+			
Pectenogammarus olivii (H. Milne Edwards, 1830)	+			
Fam. Gammarellidae Bousfield, 1977	+			
Fam. Gammaridae Latreille, 1802	+	+	+	+
Amphithopsis depressa Schiecke, 1976		+	+	
Elasmopus rapax A. Costa, 1853	+	+	+	+
Quadrimaera inaequipes (A. Costa in Hope, 1851)		+		+
Maera sodalis Karaman & Ruffo, 1971		+		
Elasmopus pocillimanus (Spence Bate, 1863)		+	+	+

Maera grossimana (Montagu, 1808)		+		+
Apohyale crassipes (Heller, 1866)	+	+	+	+
Ptilohyale eburnea (Krapp-Schickel, 1974)	+	+	+	+
Apohyale perieri (Lucas, 1846)	+	+	+	
Orchestia gammarellus (Pallas, 1766)	+	+	+	
Fam. Talitridae Rafinesque, 1815	+			
Protohyale (Protohyale) schmidtii (Heller, 1866)	+	+	+	+
Parhyale aquilina (Costa, 1857)	+	+	+	+
Marinohyalella richardi (Chevreux, 1902)		+		
Protohyale (Protohyale) grimaldii (Chevreux, 1891)		+		
Parhyale plumicornis (Heller, 1866)			+	
Anthura gracilis (Montagu, 1808)				+
Dynamene edwardsi (Lucas, 1849)	+	+	+	
Dynoides elegans (Boone, 1923)	+	+	+	+
Cymodoce delvarii Khalaji-Pirbalouty, Bruce & Wägele, 2013		+		
Heterotanais oerstedii (Krøyer, 1842)	+	+	+	+
Perforatus perforatus (Bruguière, 1789)			+	
Pagurus Fabricius, 1775		+		
Cnidaria				
Anemonia sulcata (Pennant, 1777)				
Echinodermata				
Amphiura chiajei Forbes, 1843		+	+	
Amphipholis squamata (Delle Chiaje, 1828)			+	+
Mollusca				
Ischnochiton rissoi (Payraudeau, 1826)			+	
Aplysia punctata (Cuvier, 1803)		+		
Clanculus corallinus (Gmelin, 1791)		+		
Rhyssoplax olivacea (Spengler, 1797)	+			
Bittium reticulatum (da Costa, 1778)	+	+	+	+
Bittium latreillii (Payraudeau, 1826)	+	+		+
Cerithium renovatum Monterosato, 1884				
Cerithiopsis diadema Monterosato, 1874		+		
Similiphora similior (Bouchet & Guillemot, 1978)		+	+	
Melarhaphe neritoides (Linnaeus, 1758)		+		
Alvania cimex (Linnaeus, 1758)		+		
Alvania discors (T. Brown, 1818)	+			
Alvania mamillata Risso, 1826				+
Rissoa lia (Monterosato, 1884)				+
Rissoa lilacina Récluz, 1843		+		
Rissoa membranacea (J. Adams, 1800)				+
Rissoa similis Scacchi, 1836		+		
Rissoa splendida Eichwald, 1830				
Columbella rustica (Linnaeus, 1758)	+	+		+
Tritia louisi (Pallary, 1912)				+
Aplus coccineus (Monterosato, 1884)		+		
Aplus dorbignyi (Payraudeau, 1826)		+		
Enginella leucozona (R. A. Philippi, 1844)	+			
Pisania striata (Gmelin, 1791)		+	+	+
Conus ventricosus Gmelin, 1791		+		+
Isara cornea (Lamarck, 1811)		+		
Episcomitra cornicula (Linnaeus, 1758)		+		
Muricopsis cristata (Brocchi, 1814)		+		
Ocenebra edwardsii (Payraudeau, 1826)		+	+	+

Ocenebra ingloria (Crosse, 1865)		+		+
Ocenebra paddeui (Bonomolo & Buzzurro, 2006)		+		
Turbinella sp. Lamarck, 1799		+		
Gibberula crisae Ortea & Moro, 2020	+			
Parthenina clathrata (Jeffreys, 1848)		+		
Patella aspera Röding, 1798	+	+	+	
Patella caerulea Linnaeus, 1758	+	+	+	+
Patella rustica Linnaeus, 1758	+	+	+	
Patella vulgata Linnaeus, 1758	+	+	+	
Diodora gibberula (Lamarck, 1822)		+		
Diodora italica (Defrance, 1820)	+			
Puncturella piccirida Palazzi & Villari, 2001		+		
Calliostoma gualterianum (R. A. Philippi, 1848)		+		
Calliostoma zizyphinum (Linnaeus, 1758)				+
Gibbula ardens (Salis Marschlins, 1793)				+
Gibbula turbinoides (Deshayes, 1835)		+	+	+
Phorcus turbinatus (Born, 1778)		+		
Steromphala adriatica (R. A. Philippi, 1844)	+			
Steromphala pennanti (R. A. Philippi, 1851)		+	+	
Steromphala umbilicalis (da Costa, 1778)		+		+
Steromphala varia (Linnaeus, 1758)			+	+
Arca noae Linnaeus, 1758			+	
Idas cylindricus Pelorce & Poutiers, 2009		+	+	
Mytilaster marioni (Locard, 1889)			+	
Lithophaga lithophaga (Linnaeus, 1758)			+	
Modiolula phaseolina (R. A. Philippi, 1844)				
Modiolus adriaticus Lamarck, 1819			+	
Modiolus barbatus (Linnaeus, 1758)	+	+	+	
Musculus costulatus (Risso, 1826)	+	+	+	+
Mytilus edulis Linnaeus, 1758	+		+	+
Mytilus galloprovincialis Lamarck, 1819	+	+	+	+
Perna perna (Linnaeus, 1758)			+	
Isognomon legumen (Gmelin, 1791)		+	+	+
Anomia ephippium Linnaeus, 1758		+		
Talochlamys multistriata (Poli, 1795)	+			
Arca noae Linnaeus, 1758			+	
Idas cylindricus Pelorce & Poutiers, 2009		+	+	

THE STUDIES ON ABIOTIC STRESS INTERACTION WITH VERMICOMPOST IN CULTIVATED PLANTS IN SOLANACEAE

Aysun CAVUSOGLU[1]**, Halil SAMET*[2]

*[1]*Kocaeli University, Faculty of Agriculture, Department of Plant Protection, 41285, Kocaeli, Türkiye*
[2]*Kocaeli University, İzmit Vocational School, Department of Crop and Animal Production,41285, Kocaeli, Türkiye*

**Corresponding author e-mail: cavusoglu@kocaeli.edu.tr*

ABSTRACT

A fertilizer is a material derived from natural or synthetic origin that is mostly applied to soil or to plant tissues to supply plant nutrients. With increasing concerns about environmental pollution and human health, trust in synthetic fertilizers has decreased and the tendency to use natural fertilizers has increased. One of the organic fertilizers is vermicompost. This fertilizer is obtained due to worms processing in the soil, or the nutrients presented to them, and has been included in many scientific studies. Most of these studies are on the Solanaceae family members, which are considered as vegetables and medicinal-aromatic-ornamental plants. While most of these studies were conducted on plant productivity, some were carried out to determine whether it protects the plant against biotic or abiotic problems. The studies on plant productivity measure and evaluate factors such as yield, plant height, flowering, fruit set, root, stem, tuber, and leaf development. In terms of plant health, besides biotic factors, the effects of edaphic factors such as salinity and nutrient deficiency, environmental factors such as drought, undesirable cold or hot climate conditions, and their interaction with vermicompost fertilizers have been investigated. In this review study, the data on abiotic stress interactions with vermicompost usage regarding the forms, times and results of applications on Solanaceae family cultivars such as tomato, pepper, eggplant, potato, and petunia were focused and compiled.

Keywords: Abiotic Stress, Plant Protection, Vermicompost, Organic Fertilizer, Solanaceae, Vegetable, Ornamental Plants

INTRODUCTION

Solanaceae family contains approximately 100 genera and 2500 species, and the species mostly occur on all temperate and tropical continents (D’Arcy, 1991; Olmstead & Bohs, 2007; Olmstead et al., 2008). The family includes wild and most important agricultural cultivated species, which have economic importance in human nutrition, and medicinal-aromatic-ornamental plant sectors. In the cultivation steps, fertilization is one of the most common tasks a producer does. Farmers mostly use this fertilization process to improve yield. However, as a result of the studies and observations, it has been observed and demonstrated that effective fertilization overcomes abiotic problems such as drought, soil salinity, and adverse weather conditions in addition to macro and microorganism-related biotic problems. For this reason, although many types of fertilizers are used and have the potential to be used, organic fertilizers have begun to come to the fore in terms of environmental pollution and human health. One of the organic fertilizers that has been produced in businesses that have made significant investments in recent years is vermicompost. This fertilizer, which is obtained as a result of worms processing the soil or the nutrients given to them, has been included in many scientific studies. Some of the studies were on the growth effect on fruits (Pawar et al., 2020; Geiklooi & Shirmohammadi, 2013), vegetables (Espinosa-Palomeque et al., 2020; Ali & Kashem, 2018), field crops (Prajapati et al., 2018; Ding et al., 2021) and on ornamental plants (Esringü et al., 2022; Kumar et al., 2022). Some studies about growth and yield have shown that vermicompost and inorganic fertilizers are successful when applied together (Ali & Kashem, 2018; Prajapati et al., 2018). In some studies, liquid vermicompost was applied to obtain an effect on growth and yield (Elumalai et al., 2013; Awadhpersad et al., 2021).

In this study, the studies reviewed that have been carried out on dealing with adverse growing conditions that are problems in plants by using vermicompost as abiotic factors. For this aim the data from the studies obtained regarding the forms, times and results of vermicompost applications on cultivated Solanaceae family plants such as tomato, pepper, eggplant, potato, and ornamental plants such as petunia were focused and compiled.

THE STUDIES ON ABIOTIC STRESS-VERMICOMPOST INTERACTION IN SOLANACEAE

Salinity-Vermicompost

Undoubtedly, salinity has become a major problem in agricultural lands. The physiological stressor can reduce water availability (Cavusoglu, 2023) and can cause the absorption of toxic ions while causing a decrease of absorption of essential elements (Acosta-Motos et al., 2017).

Tomato (*Solanum lycopersicum* L.) is one of the most known and consumed species of vegetables worldwide in Solanaceae which is a glycophytic species and is affected by salt stress in different degrees of salt concentration. In a study on the preventive effect of vermicompost against salt stress in tomatoes, vermicompost leachate was applied to a cultivar of fifteen-day-old tomato seedlings in aerated hydroponic culture in a growth chamber under 16 hours photoperiod. In this study, which aimed to show the effect of vermicompost applications before and concomitantly of 150 mM NaCl stress with nutrient solution, the application of before salt stress was found more effective than the second application time. According to the data, in the tomato, vermicompost leachate application abolished the harmful effect of salt stress on growth via decreasing Na^+ accumulation partly (Benazzouk et al., 2018).

Potato is one of the top ten major crops around the world. The plant is grown nearly in all countries and some of the fields face abiotic problems such as organic matter deficiency, drought, and salinity. A study was conducted with vermicompost and vermiwash to obtain their effect on potato growth under salinity stress. For this purpose, vermicompost and vermiwash were applied at three doses each and salinity at 15, 20, and 25 mM NaCl levels. The study showed that vermicompost and vermiwash have ameliorative effects on the growth of tuber, plant habitus, and tuber characteristics under saline conditions (Pérez-Gómez et al., 2017).

In a study about seedling substrates and salt stress, three different substrates, including 100% vermicompost and %50 vermicompost with peat, and only peat were used in the first step of obtaining pepper (*Capsicum annuum* L.) seedlings. Salt stress was applied to the seedling at the second step which was at the transplanting site. Briefly, the study showed that vermicompost and its mixture with peat are the best media in the nursery period. In the second step, transplanted seedlings from the two substrates showed better results under saline conditions than other used peat substrate (Kaciu et al., 2011).

Drought-Vermicompost

Drought is another environmental abiotic stress factor that threatens crop production at unexpected times and in unexpected ways. Drought causes significant yield losses worldwide in areas where irrigation water is limited.

Ebrahimi et al. (2021) used vermicompost in two levels (0 and 1500 g/m^2), pistachio and date palm biochar (0, 500 g/m^2 each) alone or in combination to find out the effect of them on irrigation water deficiency at three levels (50, 75 and 100% of plant water requirement) in eggplant (*Solanum melongena*) under the field condition. Four months after planting; chlorophyll a and b, antioxidant capacity, malondialdehyde and antioxidant enzyme activity, plant nutrients in the plant tissue, yield components, and water use efficiency were determined. The results showed that vermicompost and biochar have positive effects on eggplant growth and yield. Their usage increased N, P, K, Fe, and Mn in plant leaves. Their usage at 50% plant water requirement increased water use efficiency. The results are important especially in arid and semi-arid conditions.

Petunia is a Solanaceous, spring and summer plant that sometimes has to be planted under soil conditions such as rainless, infertent and limited-irrigation in gardens. A study was conducted with vermicompost and two other organic fertilizers to find out the effect of them on the petunia under drought conditions. The used drought rates were 100%, 50%, 25% field capacity of water. The results of the study showed that the effects of the vermicompost treatments on some parameters that are important for petunia growth can be improved under different irrigation regimes via improving water holding capacity (Goldani & Kamali, 2016).

Plant Nutrient Deficiency-Vermicompost

Plant nutrition is a key component in agricultural plant production. Nutrient deficiency effects lots of physiological steps on the road to yield. For this reason, organic or inorganic fertilization is done.

In a study conducted on the effect of vermicompost application in increasing doses to pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) P and K contents, vermicompost quantities (0, 3, 5, and 7%) were applied to the soil under the controlled laboratory condition at viol. The P and K levels of the 40-day-old test plant were analyzed after drying at 65°C in the oven. The results showed that both elements increased in both plant species with increasing vermicompost levels (Bellitürk et al., 2017).

Petunia spp. is a pot or garden flower, included in the Solanaceae family. Petunia hybrids are marketed and grown fondly around the world. The ornamental plants sometimes have to be planted on infertile soils in the garden or pots. A study was carried out with vermicompost, inorganic NPK fertilizer, *Azospirillum* sp., and phosphate solubilizing bacterium added to soil with low nutrient content. The vermicompost and others that are used in this study were applied singly or in combination. As a result of the study, when the infertile soil (control) group and other applications were evaluated together, it was seen that the highest growth values were reached when inorganic and two bio-fertilizers were added to vermicompost (Moghadam & Shoor, 2013).

It is a known fact that fertilization is required in most cases when growing tomatoes in the fields because of poor soil conditions. In a study, vermicompost was applied to the soil at rates of 0/1, 1/1, ½, 1/3, ¼ and 1/5 (v/v) under a greenhouse condition. A hundred days after planting, measurement showed that plant height, marketable tomato yield and titratable acidity were found as the best at higher vermicompost rates. The results indicate that soils need enrichment and vermicompost may be a good option for this (Gutiérrez-Miceli et al., 2007).

Temperature-Vermicompost

Unexpected high, low, and unwanted changes in temperature affect the plant's health and yield. As the plant moves away from the optimum temperature, it reduces its physiological function and yield. Studies, besides Solanaceae, are showing that these temperature-related negative reactions can be reduced with vermicompost.

Because it originated from tropical and mild-temperate regions, most Solanaceous plants sensitive to too cold and hot. In a study (Chinsamy et al., 2014), tomato seedlings treated with vermicompost leachate (1:10 v/v) under different temperatures (10, 15, 20, 25, and 30°C) at perlite substrate enriched with Hoagland's solution were used. It has been revealed that vermicompost has positive contributions to leaf number, stem thickness, plant leaf area, chlorophyll b, proline, and total sugar properties at both the lowest (10°C) and the highest (30°C) temperature. The study clearly emphasized that vermicompost leachate has growth-enhancing effects on tomato seedlings under non-optimal temperatures.

Since studies on vermicompost focus on other stress factors such as nutrient deficiency and salinity, few studies could be revealed on temperature differences in solanaceous plants.

CONCLUSION

The fertilizers applied to the rooting media or upper part of the plant not only have plant nutrition properties but also have the function of alleviating the effects of abiotic factors that begin to disturb the plant by moving away from the optimum. When evaluated from this perspective, vermicompost, whether in solid or liquid form, appears to be an environmentally friendly, relatively cheaper, easy-to-use, and suitable option for organic agriculture in Solanaceae plants. The latest studies showed that vermicompost was effective in eliminating abiotic stress factors that may occur in Solanaceae plants. But many other stress factors in plants that may occur in different places and at different times still need to be studied.

REFERENCES

Acosta-Motos, J. R., Ortuño, M. F., Bernal-Vicente, A., Diaz-Vivancos, P., Sanchez-Blanco, M. J., & Hernandez, J. A. (2017). Plant responses to salt stress: adaptive mechanisms. *Agronomy*, *7*(1), 18. doi: 10.3390/agronomy7010018

Ali, S., & Kashem, M. A. (2018). Effect of vermicompost on the growth and yield of cabbage. *Journal of Agricultural Engineering and Food Technology*, *5*(1), 45-49.

Awadhpersad, V. R. R., Ori, L., & Ansari, A. A. (2021). Production and effect of vermiwash and vermicompost on plant growth parameters of tomato (*Lycopersicon esculentum* Mill.) in Suriname. *International Journal of Recycling of Organic Waste in Agriculture*, *10*(4), 397-413. doi: 10.30486/IJROWA.2021.1911898.1148

Bellitürk, K., Adiloğlu, S., Solmaz, Y., Zahmacıoğlu, A., & Adiloğlu, A. (2017). Effects of increasing doses of vermicompost applications on P and K contents of pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.). *Journal of Advanced Agricultural Technologies Vol*, *4*(4), 372-375. doi: 10.18178/joaat.4.4.372-375

Benazzouk, S., Djazouli, Z. E., & Lutts, S. (2018). Assessment of the preventive effect of vermicompost on salinity resistance in tomato (*Solanum lycopersicum* cv. Ailsa Craig). *Acta Physiologiae Plantarum*, *40*, 121. doi: 10.1007/s11738-018-2696-6

Cavusoglu, A. (2023). Salinity stress as an abiotic factor at germination stage on dry bean (*Phaseolus vulgaris* L.) cultivars. *Current Trends in Natural Sciences*, *12*(23), 17-27. doi: 10.47068/ctns.2023.v12i23.002

Chinsamy, M., Kulkarni, M. G., & Van Staden, J. (2014). Vermicompost leachate reduces temperature and water stress effects in tomato seedlings. *HortScience*, *49*(9), 1183-1187. doi: 10.21273/HORTSCI.49.9.1183

D'Arcy, W.G. (1991). The Solanaceae since 1976, with a review of its biogeography. Pp. 75–138 in: Hawkes, J.G., Lester, R.N., Nee, M. & Estrada, N. (eds.), Solanaceae III: Taxonomy, Chemistry, Evolution. Royal Botanic Gardens, Kew

Ding, Z., Kheir, A. M., Ali, O. A., Hafez, E. M., ElShamey, E. A., Zhou, Z., ... & Seleiman, M. F. (2021). A vermicompost and deep tillage system to improve saline-sodic soil quality and wheat productivity. *Journal of Environmental Management*, *277*, 111388. doi: 10.1016/j.jenvman.2020.111388

Ebrahimi, M., Souri, M. K., Mousavi, A., & Sahebani, N. (2021). Biochar and vermicompost improve growth and physiological traits of eggplant (*Solanum melongena* L.) under deficit irrigation. *Chemical and Biological Technologies in Agriculture*, *8*, 19. doi: 10.1186/s40538-021-00216-9

Elumalai, D., Kaleena, P. K., Fathima, M., & Hemavathi, M. (2013). Influence of vermiwash and plant growth regulators on the exomorphological characters of *Abelmoschus esculentus* (Linn.) Moench. *African Journal of Basic and Applied Sciences*, *5*(2), 82-90.

Espinosa-Palomeque, B., Cano-Ríos, P., Salas-Pérez, L., González-Rodríguez, G., Reyes-González, A., Ayala-Garay, A. V., & Preciado-Rangel, P. (2020). Vermicompost on the production and nutraceutical quality of jalapeño pepper fruits (*Capsicum annuum* L.). *Terra Latinoamericana*, *38*(4), 795-803. doi: 10.28940/terra.v38i4.605

Esringü, A., Turan, M., Sushkova, S., Minkina, T., Rajput, V. D., Glinushkin, A., & Kalinitchenko, V. (2022). Influence of vermicompost application on the growth of *Vinca rosea valiant*, *Pelargonium peltatum* L. and *Pegasus patio rose*. *Horticulturae*, *8*(6), 534. doi: 10.3390/horticulturae8060534

Geiklooi, A., & Shirmohammadi, E. (2013). Effect of enriched vermicompost manure in improve of iron and zinc deficiencies and quality characteristics of peach trees. *International Journal of Farming and Allied Sciences*, *2*(21), 930-934.

Goldani, M., & Kamali, M. (2016). Evaluation of culture media including vermicompost, compost and manure under drought stress in Iranian petunia (*Petunia hybrida*). *Plant Productions*, *39*(3), 91-100. doi: 10.22055/ppd.2016.12335

Gutiérrez-Miceli, F. A., Santiago-Borraz, J., Molina, J. A. M., Nafate, C. C., Abud-Archila, M., Llaven, M. A. O., ... & Dendooven, L. (2007). Vermicompost as a soil supplement to improve growth, yield and fruit quality of tomato (*Lycopersicum esculentum*). *Bioresource Technology*, *98*(15), 2781-2786. doi: 10.1016/j.biortech.2006.02.032

Kaciu, S., Babaj, I., Sallaku, G., & Balliu, A. (2011). The influence of vermicompost on plant growth characteristics and stand establishment rate of pepper (*Capsicum annuum* L.) seedlings under saline conditions. *Journal of Food, Agriculture & Environment*, *9*(1), 488-490.

Kumar, P., Sheoran, S., & Beniwal, B. S. (2022). Growth and yield parameters of rose as influenced by different organic manures and their levels. *The Pharma Innovation Journal*, *11*(6), 394-398.

Moghadam, M. Z., & Shoor, M. (2013). Effects of vermi-compost and two bacterial bio-fertilizers on some quality parameters of *Petunia*. *Notulae Scientia Biologicae*, *5*(2), 226-231. doi: 10.15835/nsb528305

Olmstead, R.G. & Bohs, L. (2007). A summary of molecular systematic research in Solanaceae: 1982–2006. Pp. 255– 268 in: Spooner, D.M., Bohs, L., Giovannoni, J., Olmstead, R.G. & Shibata, D. (eds.), Solanaceae VI: Genomics Meets Biodiversity. Proceedings of the

Sixth International Solanaceae Conference. Acta Horticulturae 745. International Society for Horticultural Science, Leuven.

Olmstead, R. G., Bohs, L., Migid, H. A., Santiago-Valentin, E., Garcia, V. F., & Collier, S. M. (2008). A molecular phylogeny of the Solanaceae. *Taxon*, *57*(4), 1159-1181. doi: 10.1002/tax.574010

Pawar, P. S., Garande, V. K., & Bhite, B. R. (2020). Effect of vermicompost and biofertilizers on growth, yield and fruit quality of sweet orange (*Citrus sinensis* L. Osbeck) cv. Mosambi. *Journal of Pharmacognosy and Phytochemistry*, *9*(4), 3370-3372.

Pérez-Gómez, J. D. J., Abud-Archila, M., Villalobos-Maldonado, J. J., Enciso-Saenz, S., Hernández de León, H., Ruiz-Valdiviezo, V. M., & Gutiérrez-Miceli, F. A. (2017). Vermicompost and vermiwash minimized the influence of salinity stress on growth parameters in potato plants. *Compost Science & Utilization*, *25*(4), 282-287. doi: 10.1080/1065657X.2017.1333932

Prajapati, V. K., Swaroop, N., Masih, A., & Lakra, R. (2018). Effect of different dose of NPK and vermicompost on growth and yield attributes of maize [*Zea Mays* (L.)] Cv. MM2255. *Journal of Pharmacognosy and Phytochemistry*, *7*(1), 2830-2832.

DETERMINATION OF FACTORS AFFECTING UNIVERSITY STUDENTS' CORN CONSUMPTION PREFERENCES

Ipek Atılgan Helvacıoğlu[1] , Cem Tokatlı[1], Beyza Kahraman[1] & Aslı Kaya[1]

[1]Trakya University, Evrenos Gazi Campus, İpsala Vocational School, Department of Laboratory Technology, Edirne / Türkiye

**Corresponding Author: ipekhelvacioglu@trakya.edu.tr*

ABSTRACT

In this survey conducted by Trakya University İpsala Vocational School Laboratory Technology Program students with 199 university students, 7 survey questions were asked and the participants were asked about their corn consumption preferences. Among the corn consumption types presented, the participants preferred popcorn the most with a rate of 35.2%, followed by corn chips with 22.6%. 42.7% of the participants stated that they consume corn monthly, while 35.2% stated that they consume it weekly. In the question asked about the value of corn in terms of human nutrition, 50.3% stated that they did not have any information, and the closest rate to this was 39.7%, which indicated that it was a healthy food as far as they followed from the media. When purchasing any corn product, 40.2% of the participants stated that they cared about the information about the production method or processing of the product, and the closest to this was their brand preference with 25.1%. 72.4% of the participants wanted the corn product they consumed to contain no additives. In the question about the future situation of corn, 49.2% of the participants stated that they preferred that the interest in corn would increase, while 19.1% of the participants thought that production would decrease due to the risk of drought. Again, in the question about the consumption rate of corn oil in the future, 43.6% of the participants stated that consumption would increase if its price was lower than sunflower oil.

Keywords: Corn, Consumption Preference, University Students

INTRODUCTION

The corn plant is still one of the six grains that feed the world's population. Corn, which has the largest cultivation area after wheat and barley among grains in our country, is successfully produced as the main product and second product. After the 1980s, significant increases were recorded in corn production in Turkey. The reason for the increase in corn yield is that the state encourages corn production, producers implement modern corn production techniques, expand the use of hybrid seeds, shift corn production to irrigated areas and ensure the use of fertilizers at certain levels. Although the production reached 5.95 million tons in 2014 in our country, the ratio of production to consumption was 90%. 78% of the corn produced in our country is used in the feed industry and 15% is used in the starch sector. In recent years, corn cultivation for silage purposes has become quite widespread, especially as a second crop

Corn has a very important place in the world agricultural products market in terms of both production and demand and foreign trade. In addition to being largely used as animal feed, its use for human nutrition further increases its importance. In Turkey, corn is the product that is very common in production and has the highest production share after wheat and barley in the grain group (Akkurt and Demirbaş, 2021)

The corn plant is divided into seven varieties. The most important varieties of corn, dent and hard corn, are used intensively in human and animal nutrition. In addition, special corn types such as popcorn (Zea mays L. everta), sweet corn (Zea mays L. saccharata sturt.) and waxy corn (Zea mays ceratina) can be used as a snack by popping, boiled or canned. Popcorn, a traditional snack food in our country, has been grown on approximately 9000-10,000 hectares of land in recent years and is especially enjoyed by children. Sweet corn is consumed boiled and canned, especially in coastal regions, especially in the Mediterranean and Aegean regions. The demand for waxy corn is also increasing day by day throughout the country.

The main areas of use of corn are human food and industrial raw material. Corn stalks and leaves are used as animal feed, especially in the form of silage, and the stalks and leaves are also used in the industry for paper production and small-scale wicker crafts. In addition to its main areas of use, corn is also consumed as a snack in special variety groups. Corn's special areas of use include the oil and sweetener sector and biofuel-bioethanol production.

Popcorn is used specifically as a snack, its difference from other snack foods is that it contains low calories and fat, and high carbohydrates (Hansen, 2012). Özkan (2007) stated that one of the reasons popcorn is preferred in nutrition is the vitamins and minerals it contains.
It has been reported that it is a good diet product due to its ability to keep you full and absorb stomach acid (Ülger, 1998). When the energy value of popcorn is examined, it is reported to be equivalent to two eggs on average. Nguyen et al. (2012) reported in a study they conducted that popcorn provides a feeling of satiety due to its low calorie value and the energy it provides from whole grain corn, and that it can be an important choice in body weight control thanks to this feature.

Popcorn has a special place among other corn variety groups with its popping feature and its ability to be used as a snack by turning it into popcorn and its rich nutritional content. The demand for popcorn is increasing in our country, especially because it is consumed with pleasure by children and preferred as a snack by moviegoers. Sweet corn is among the special corn types and its production and consumption are increasing rapidly due to its nutritional content, flavor and different areas of use. Purple waxy corn, whose consumption trend is rapidly increasing for modern consumers in the world due to its health benefits, is also increasing rapidly in our country (Öztürk et al., 2019).

MATERIALS AND METHODS

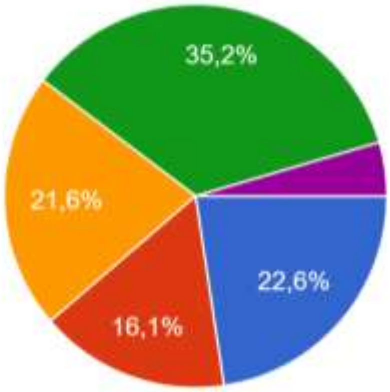
In this study conducted by Trakya University İpsala Vocational School Laboratory Technology program students with students from different universities in Turkey, random sampling method was used and 7 survey questions were directed to 199 students studying at different faculties and vocational schools and the participants were asked questions about their corn consumption preferences. In this study conducted with face-to-face survey method, the students were asked about their corn consumption preferences, corn consumption frequency, whether they have knowledge in terms of human nutrition, their perspective on corn additives and whether they have knowledge about corn production techniques.

.

RESULTS AND DISCUSSION

In this research conducted by Trakya University İpsala Vocational School Laboratory Technology program students using a face-to-face survey method, the first question asked to the students was corn consumption preferences. The participants' answers are shown in Fig.1 below.

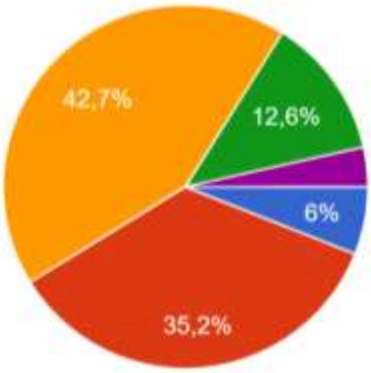
Fig.1. Corn consumption preferences of students from different universities in Turkey



According to the table in Figure 1, popcorn ranks first in students' corn consumption preferences with a rate of 35.2%. This is followed by corn chips with a close gap of 22.6% and corn cobs with a rate of 21.6%. Corn flakes were also preferred by 16.1%. The rest of the students said that they did not consume corn. In general, it was observed that there was no significant difference between the students' corn consumption preferences due to the closeness of the rates.

The second question asked to the students was the frequency of corn consumption. The participants' answers are shown in Figure 2.

Fig. 2. Frequency of corn consumption of students from different universities in Turkey



According to Fig.2, the frequency of students' corn consumption was determined as 42.7% once a month. This was followed by 35.2% once a week, 12.6% annual consumption and 6% daily consumption.

The answers given by the students to the third question, which asked about the value of corn in terms of human nutrition, are shown in Fig.3.

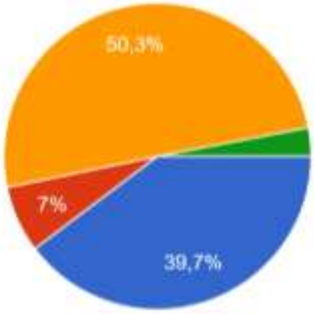
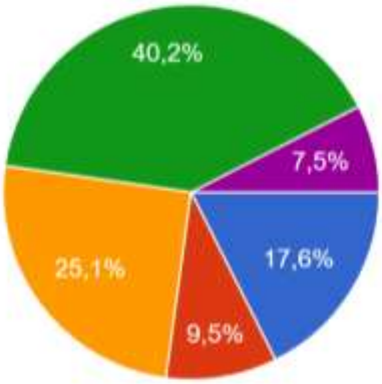


Fig.3. Knowing the value of corn in human nutrition

According to Fig.3, 50.3% of the students stated that they had no information about the value of corn in human nutrition. 39.7% of the students said that they believed that corn had an important place in human nutrition, while 7% said that they thought it was likely to be harmful. The answers of the students to the question asking about the factors they pay attention to when buying any corn product are shown in Fig.4.

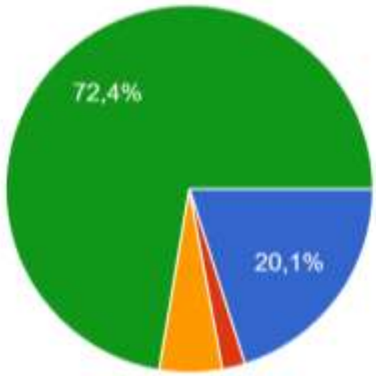
Fig.4. Factors they pay attention to when buying corn products



Students stated that they pay attention to whether the corn variety they desire is GMO at a rate of 40.2%, make a brand preference at a rate of 25.1%, care about nutritional value at 17.6%, and calorie content at 9.5%.

The 5th question asked to students attempted to learn which additives in corn products would be acceptable to them. The students' answers to this question are shown in Fig. 5.

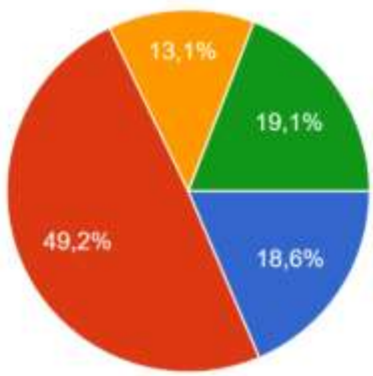
Fig.5. Which additives in corn products are acceptable?



When looking at Fig.5, the vast majority of the students participating in the survey, 72.4%, did not want any additives and wanted to consume the product completely natural. 20.12% of the students thought that preservatives would be acceptable in corn products. Colorants and sweeteners, which were among the other options asked, were the substances preferred by the students in very small proportions.

The response rates given by the students to the 6th question, which asked about the future role of corn, are shown in Fig.6.

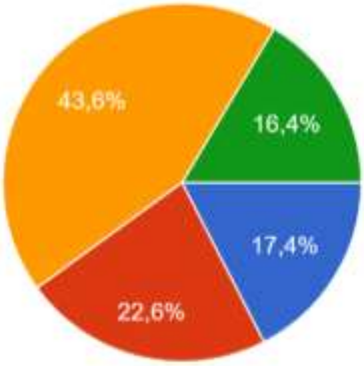
Fig.6. The future role of corn



49.2% of the students who participated in the survey thought that corn oil would be consumed much more in the future, 19.1% thought that corn production would decrease significantly, 18.6% thought that corn would grow only in the oil industry and 13.1% thought that it would be used only for silage.

The situation of these two vegetable oil sources in the future was asked in Question 7, as corn oil is considered to be a competitor to sunflower oil by the students who prepared the survey. The answers of the students who participated in the survey are given in Fig.7.

Fig.7. Comparison of the future situations of sunflower oil and corn oil



43.6% of the students stated that corn oil would be preferred more than sunflower oil if its price was lower, 22.6% thought that sunflower oil would always be preferred more, 17.4% believed that corn oil would be preferred more, and 16.4% stated that sunflower would always be preferred more than other oil types.

CONCLUSIONS

In this study conducted by students of Trakya University İpsala Vocational School Laboratory Technology program with students from different universities in Turkey, it was tried to understand the students' opinions about corn consumption. It was stated that although the students did not have much knowledge about corn cultivation, they were informed about GMO products and generally followed them on social media. Students who are aware of the importance of consuming healthy products stated that they prefer to consume popcorn especially when they socialize, even if occasionally.

Although it is preferred for consumption as a snack, the students thought that corn oil would be preferred if it is more economical than sunflower oil.

REFERENCES

Cengiz, R.,2016. Türkiye'de Kamu Mısır Araştırmaları. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 2016, 25 (Özel sayı-1):304-310 Derleme (Rewiev)

ÖZTÜRK,A., Erkan ÖZAT,E., Şekip ERDAL,Ş., Mehmet PAMUKÇU, M.,2019. Türkiye'de Özel Mısır Tiplerinin Kullanımı ve Geleceği.Uluslararası Doğu Akdeniz Tarımsal Araştırma Enstitüsü Dergisi, 2(1): 75-90, 2019 75 Derleme

Akkurt, E. and Demirtaş, N., 2021. Türkiye'de Mısır Üretiminde Kendine Yeterliliğin Değerlendirilmesi XV. IBANESS Congress Series on Economics, Business and Management – Plovdiv / Bulgaria XV. IBANESS İktisat, İşletme ve Yönetim Bilimleri Kongreler Serisi – Plovdiv / Bulgaristan May 29-30, 2021 29-30 Mayıs 2021 798

THE EFFECT OF SEWAGE SLUDGE USE ON SAME SOIL PROPERTIES AND EROSION PARAMETERS IN ERODED PASTURE AREAS IN KARAPINAR

Ayşen AKAY1 Osman MÜCEVHER2 Salih BİTGİ2 Serap ULUTAŞ3 Ramazan ACAR4 Şükrü DURSUN5 Serdar KOYUNCU6 Mehtap ÖZTEKİN7

1University of Selcuk, Faculty of Agricultural, Department of Soil Science and Plant Nutrition,42130, Konya,Türkiye
2 Directorate of Soil, Water and Combating Desertification Research Institute, Konya, Türkiye
3 State Hydraulic Works 4th Regional Directorate, Konya, Türkiye
4 Selcuk University, Agriculture Faculty, Field Crops Department, Konya, Türkiye
5 Konya Technical University, Faculty of Engineering and Natural Sciences, Department of Environmental Engineering, Konya, Türkiye
6 Metropolitan Municipality KOSKİ General Directorate Konya, Türkiye
7 Directorate of National Botanical Garden of Türkiye, Ankara, Türkiye

Corresponding author email: aakay@selcuk.edu.tr

ABSTRACT

Konya Domestic Wastewater Treatment Plant produces approximately 150 tons/day of stabilized domestic treatment sludge. Disposal of the existing treatment sludge is a problem. In this study, the effect of sewage sludge in terms of soil organic matter, pH, salinity, aggregate formation, erosion prevention and soil improvement on eroded, weak and degraded pasture soils in Karapınar was investigated. For this purpose, sewage sludge containing 80% dry matter was applied to the plots at 0 (Control), 1, 2, 4 and 8 tons/da (DM) by two different methods as raking into the 0-5 cm layer of the soil and sprinkling on the soil surface. Then, soil samples were taken from the treatment plots for 3 years. According to the data obtained at the end of the experiment, it was determined that the treatments increased the soil organic matter especially in the 0-5 cm soil depth and this increase became more effective as time passed. The effect of the treatments on soil salinity partially increased over time. The average value of 0.23 mmhos/cm before the application in the first year was 0.34 mmhos/cm at the end of the fourth year. The average values of water saturation, stability index and mechanical stability index showed significant increases compared to the first sampling year. It was observed that sewage sludge applications increased soil organic matter, increased structural stability, may be effective in preventing sediment transport and consequently increased erosion resistance and resistance of soils to erosion. In the study, it was concluded that 1 ton/da can be applied in eroded weak pasture soils with a rainfall of at least 250 mm, in accordance with the regulation on the use of sewage sludge in soil, once every three years for the ecological conditions in this region, by mixing it into the soil to a depth of 0-5 cm.

Keywords: Erosion, Sewage Sludge (Biosolids), Aggregate, Karapinar, Degraded Pasture Lands.

INTRODUCTION

Due to the growth of cities and the increase in population, there is a significant increase in the amount of sewage sludge(SS) generated from wastewater treatment plants. Among the disposal methods of SS, the most cost-effective method is land disposal. However, land disposal of SS can cause serious problems due to salinity and heavy metals in the sludge. Although the content of SS (biosolids) varies from region to region and depending on the type of treatment, it can be introduced into the soil in a controlled manner because it is generally rich in organic matter, nitrogen and phosphorus (Hakerler, 1980). Biosolids are used in severely damaged marginal areas to restore the soil to a productive state or to establish vegetation to prevent soil erosion. Soil losses due to erosion decrease as the amount of biosolids applied increases. This effect is explained by the positive effect of biosolids on soil aggregation and infiltration as well as water retention and surface roughness (Bilgin et al., 2002). Sewage sludge added to loosely textured sandy soils increases the water holding capacity of these soils (Ayvaz, 2000), allows nutrient exchange and retention, increases the number and activity of soil microorganisms (EPA, 1994). In areas that are severely affected by erosion and where soil depth is low, biosolids application prevents erosion with its physical effect and then increases the emergence and development of seeds in the environment and can rapidly provide the formation of natural vegetation (İşçi, 2006).

The physical, chemical properties and biological productivity of sandy loam textured soils exposed to wind erosion in semi-arid areas are low. What should be done to improve such areas is to increase the organic matter content in the soil. Due to the organic matter content of SS, if it is used in areas exposed to erosion in arid areas, it will have the effect of improving the population of pasture plants as well as improving the soil. Therefore, due to its soil covering and erosion prevention effect, it was aimed to carry out this study and to determine the optimum application dose of SS.

MATERIALS AND METHODS

Material

Research location: The research is located in Karapınar district of Konya province in Turkey, which has 465.913 hectares of land dune with mild to very severe wind erosion problems. In Karapınar Desertification and Erosion Research Center, the study was conducted in the “No. 1 eroded, weak, fragile, degraded Yenice Pasture area” shown in Figure 1. This area constitutes 22.1% of the wind erosion area at the country level.

Figure 1. Location image of the trial site

The climate of the region is described as semi-arid continental, with dry and hot summers and cold and rainy winters. The average precipitation is 275 mm, 40% of which falls in the winter months and the average precipitation from July to September is 15 mm (Şimşekli, 2012).

In the project area, the high temperature in the summer months and the low humidity in the soil profile throughout the year reduce the amount of organic matter in the soil. As a result, it has a negative impact on the physical and chemical quality of the soils(Bot and Benites, 2005). These reductions in vegetation due to the temperature difference cause a decrease in the organic matter cycle in the soil. This plays an effective role in increasing desertification and wind erosion. The soils in Karapınar are generally of alluvial origin formed on old lake deposits. The soil series in Karapınar are presented in Figure 2. In our study, the soils of the area where SS was applied were of sandy loam texture.

Figure 2. Soil series in Karapinar (Groneman, 1968; Akça, 2001)

Characteristics of the Sewage Sludge Used in the Study: Sewage sludge (biosolids) from Konya domestic wastewater treatment plant is dewatered after digestion in airless environment. In 2011, a total of 47.728 tons of sludge (24.23% dry matter(DM)) was dewatered and removed from the system. Samples taken from these dewatered stabilized sewage sludges are regularly analyzed every month at TÜBİTAK-MAM ÇE Research Laboratory. The SS used in the project was also analyzed in the same laboratory and the analysis results were found to be in compliance with the Regulation on the Use of Treatment Sludge in Soil dated 03.08.2010 (Table 1). Bio-sludge sample number 2, whose analysis results are shown in Table 1, was selected to be used in the study.

Preparation and application of sewage sludge (biosolids): In the study, stabilized SS from the sludge dewatering unit of Konya Domestic Wastewater Treatment Plant (on 15.08.2014) was spread as a thin layer in the surrounded U-shaped concrete lagoons. The stabilized domestic SS pile, which was in compliance with the directive as a result of the analysis procedures, was turned upside down with a mixer bucket machine and crushed with a roller in certain periods and dried in an open pile in the sun for 2 months. In this way by drying method, the dry matter content of the sludge was increased from 25% DM to 80% DM by drying method. Afterwards, the SS was grinded/shredded with a rotatiller machine and sieved with a 10 mm sieve and ready-to-use biosolids piles were formed. In November 2014, the moisture content of the SS was carried out at 100% dry matter (DM) as specified in the trial plan.

The doses of SS were applied as 0 (Control), 1, 2, 4 and 8 tons/da (DM) in two treatments (mixing with rake into the 0-5 cm layer of the soil (D main subject) and spreading on the soil surface (S main subject)). The experiment was carried out in 2 main subjects, 4 replications, 5 doses of SS in a total of 40 plots. Trial plot dimensions were 8 m*10 m = 80 m^2 and each plot was side by side with a gap of 1.5 m and the gap between the blocks was set as 2.5 m. The treatments were carried out according to the randomized block split-plot experimental design.

Table 1.Konya KOSKİ Wastewater Treatment Plant, 1 and 2 treatment sludge samples analysis results, limit values and analysis methods

Parameter / Example	Sewage Sludge		Limits	Analysis Methods
	1 no.	2 no.		
Lead (Pb mg/kg furnace dry matter)	56,2	47,06	750	EPA 6020 A (ICP-MS)
Cadmium (Cd mg/kg)	8,13	8,52	10	
Chromium (Cr mg/kg)	500	598	1000	
Cupper (Cu mg/kg)	298	261	1000	
Nikel (Ni mg/kg)	175	165	300	
Zink (Zn mg/kg)	1735	1534	2500	
Mercury (Hg mg/kg)	0,617	0,596	10	TS 2537 EN 1483
Nitrogen (TKN mg/kg)	27669	25483		SM-4500-$N_{org.}$ B
Phosphorus (P mg/kg)	10297	9834		Method of burning (Olsen et al.)
PCB (mg/kg)	<0,1	<0,1	0,8	EN 12766
PCDD/F (ng TE/kg furnace dry matter)	0,183	0,44	100	TS EN 1948/2-3
pH	6,71	6,73		TS 8332 ISO 10390
C/N (%)	7,12	7,23		D.13.Y.04.24 (İnternal method)
Moisture (%)	72,57	38,29		TS 9546 EN 12280
dry matter (DM) (%)	27,43	61,71		
Loss on Combustion (glow), Organic Matter (at 770 ^oC) (%)	51,1	43,27	> 40	TS EN 12879
Conductivity (dS/m)	2,63	3,8		ISO 11265
E. coli (EMS/g)	1,8E+05 kob/g	1,9E+06 kob/g	least 2 Log10 (%99)	ISO 16649-2

1.blok	D1	D0	D8	D4	D2	S0	S2	S1	S8	S4
2.blok	S8	S4	S1	S2	S0	D2	D8	D1	D0	D4
3.blok	D0	D2	D1	D8	D4	S2	S0	S4	S8	S1
4.blok	S0	S2	S4	S8	S1	D8	D1	D0	D2	D4

Figure 3. Experimental design

D: Mixing sewage sludge (biosolids) into the soil at a depth of 0-5 cm with a rake
S: The process of spreading sewage sludge (biosolids) on the soil surface

Sewage sludge was applied at a single application in November 2014. Subsequently, the effects of SS on soil and plants were observed for 3 years after application (in June 2015, 2016 and 2017). Therefore, biosolids were not applied every year.

Method

In the study, soil samples were taken and analyzed before the application of SS (biosolid) in October 2014 and after the application in June 2015, 2016, and 2017. Samples were taken from 0-5 cm, 5-10 cm, 10-20 cm from the trial plots and then organic matter, electrical conductivity, pH and calcium carbonate analyzes were performed on the soil samples taken (Tüzüner, 1990; Kacar, 1972). Dry aggregate percentages of erosion parameters were analyzed with the help of Rotary sieve (Chepil, 1962). As it is known, Mechanical Stability Percentage, which shows the resistance of soils against wind erosion, is also an expression of the resistance of aggregates against friction. Mechanical stability values lower than 50% indicate that these soils are not resistant to erosion. As the mechanical stability increases in terms of wind erosion, the resistance to erosion increases in that soil. As the amount of organic matter in the soil decreases, mechanical stability decreases and the ability of the soil to be eroded will increase (Okur et al 2008). Using Chepil's "Rotar sieve", the mechanical stability percentages and stability indices of the soils were calculated according to the following formula.

Mechanical Stability, % = (W_1 / W) x 100

Where; W = Amount of particles larger than 0.840 mm obtained at the end of the first sieving, %

W_1 = Amount of particles larger than 0.840 mm obtained at the end of the second sieving, %

The Stability Index, which shows the erodibility (resistance to erosion) of the soils, in terms of the erosion status of the soils, those with a stability index less than 1.5 are classified as weak and those with a stability index greater than 1.5 are classified as resistant (Çelebi 1981). Statistical analyses of the obtained data were performed using Minitab 18 package program.

RESULTS AND DISCUSSION

Organic matter: When the organic matter contents of the soil samples taken for 3 years SS applications were examined, compared to the organic matter content before the first application (2014), organic matter content increased with increasing SS application doses in both spreading and mixing treatments ($p<0.01$) (Table 2, Figure 4, 5). The highest increase values are in the 8 ton SS/da application. When the difference between the years was examined, the highest increase in organic matter was observed in the second year of the SS application (2016). While there was no difference in the organic matter content of different soil depths with the applications, significant differences were observed between SS application doses and soil depth ($P<0.01$). While the amount of organic matter increased in the first and second years of the open application, it was observed that there was a slight decrease in the third year. It is thought that this situation is due to the decomposition of organic matter in the soil over time. While the organic matter content was 0.56% on average before the SS application, it increased to 1.17% on average three years after the application.

Table 2. Effect of different doses of sewage sludge applications on the average values of soil organic matter (%) according to years ($p <0.01$)

Depth(cm)	Organic Matter(%)			
	2014	2015	2016	2017
0-5	0.61 a	1.54 a	2.12 a	2.22 a
5-10	0.49 b	0.63 b	0.84 b	0.64 b
10-20	0.58 ab	0.65 b	0.88 b	0.66 b

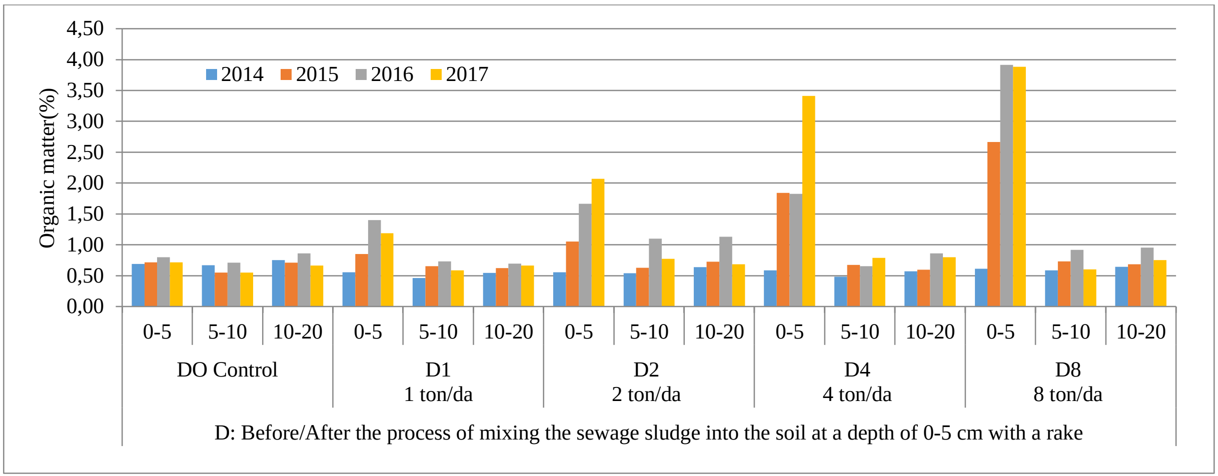


Figure 4. Effect of different doses of sewage sludge applications (D) on the average values of soil organic matter (%) content

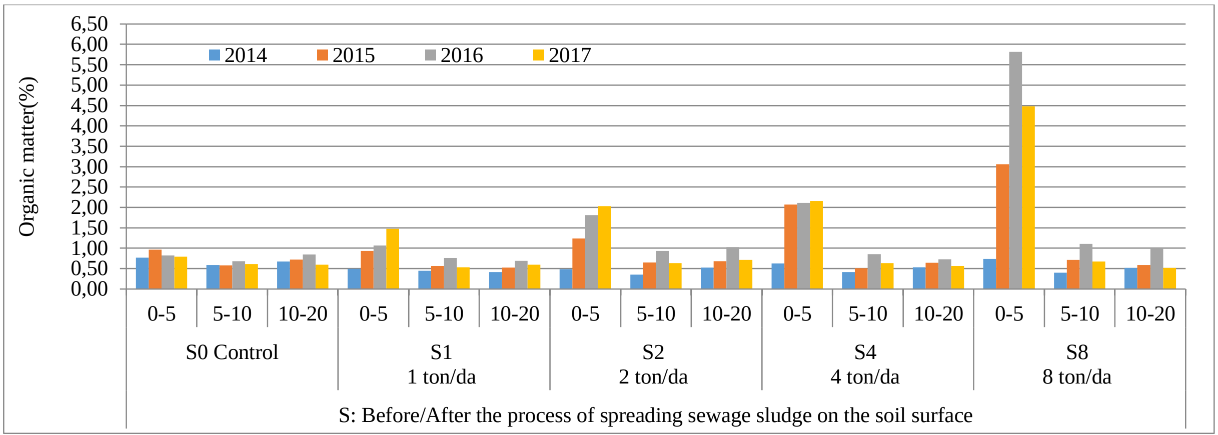


Figure 5. Effect of different doses of sewage sludge applications (S) on the average values of soil organic matter (%) content

Salinity (EC): In the experiment, there was a significant difference ($P<0.01$) in the EC values of the soil between the SS application doses. However, there was no difference between the of spreading and mixing application methods. There was also a significant difference ($P<0.01$) between the effect of SS applications and EC soil depth. Accordingly, the highest EC values were measured at 0-5 cm soil depth, while EC values decreased at 5-10 and 10-20 cm depths. In all years, EC values were below the limit value of 4 dS/m (Richards. 1954). According to the years, EC values increased approximately two times at 0-5 cm depth compared to 2014 when there was no first application (Table 3, Figure 6, 7).

Table 3. Effect of different doses of sewage sludge applications on the average values of soil EC (mmhos/cm) according to years (p <0.01)

Depth (cm)	EC*(mmhos/cm)			
	2014	2015	2016	2017
0-5	0.25 a	0.65 a	0.58 a	0.41 a
5-10	0.21 b	0.42 b	0.37 b	0.28 c
10-20	0.23 ab	0.48 b	0.43 b	0.33 b

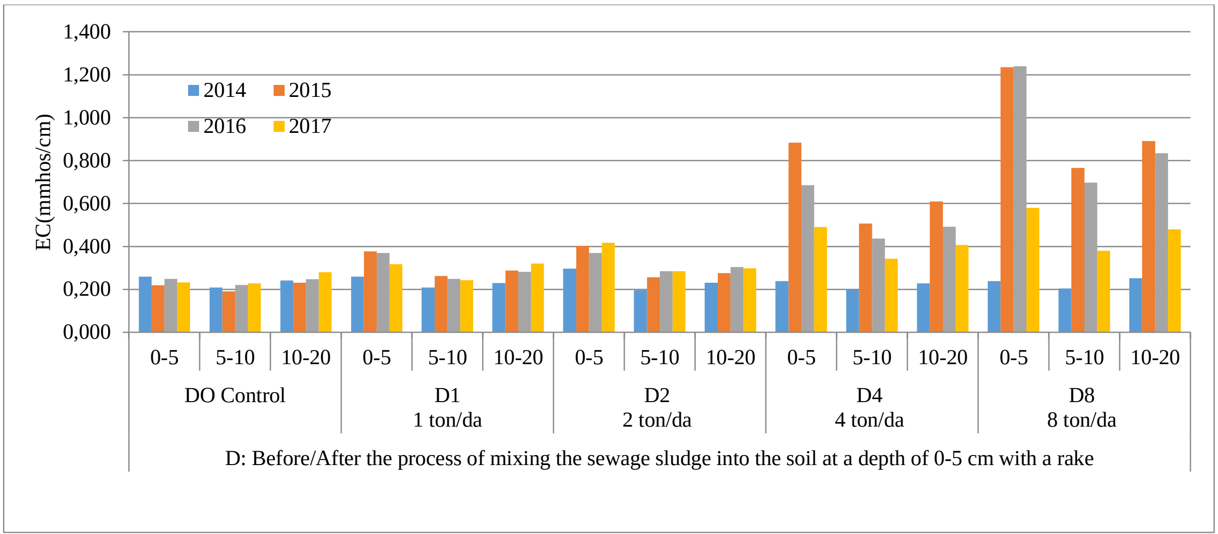


Figure 6. Effect of different doses of sewage sludge applications (D) on the average values of soil EC (mmhos/cm)

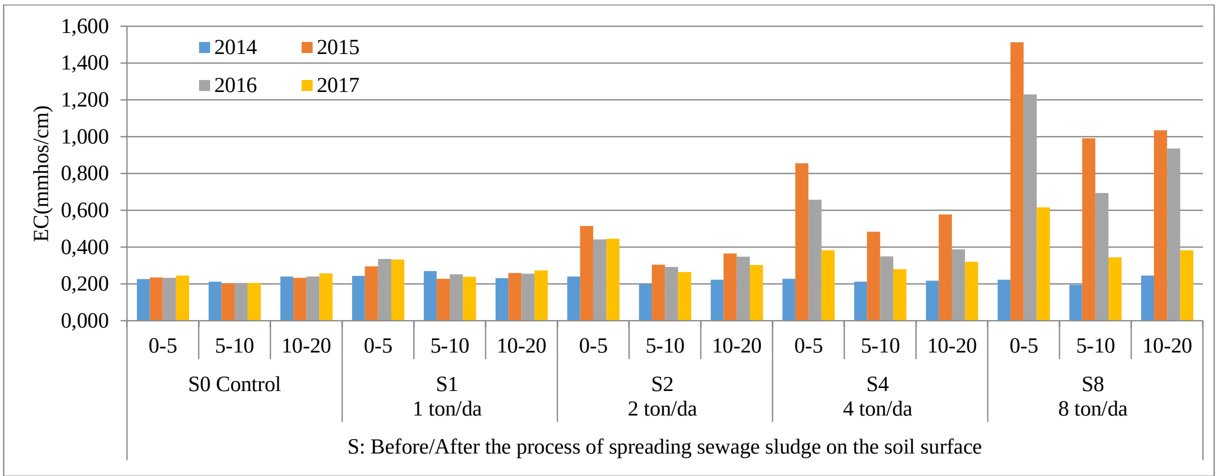


Figure 7. Effect of different doses of sewage sludge applications (S) on the average values of soil EC (mmhos/cm)

Calcium Carbonate(%): The calcium carbonate(%) content differed significantly with the SS application methods, application doses and soil depth. In 2015 and 2016 compared to 0-5 cm soil depth, lime content increased slightly at 5-10 cm depth and decreased at 10-20 cm depth (Table 4, Figure 8, 9).

Table 4. Effect of different doses of sewage sludge applications on the average values of soil $CaCO_3$ (%) according to years ($p < 0.01$)

Depth(cm)	Calcium Carbonate (%)			
	2014	2015	2016	2017
0-5	61.37 a	60.83 b	58.37 b	61.78 a
5-10	60.73 a	62.52 a	60.83 a	59.75 b
10-20	58.47 b	59.60 c	58.94 b	59.39 b

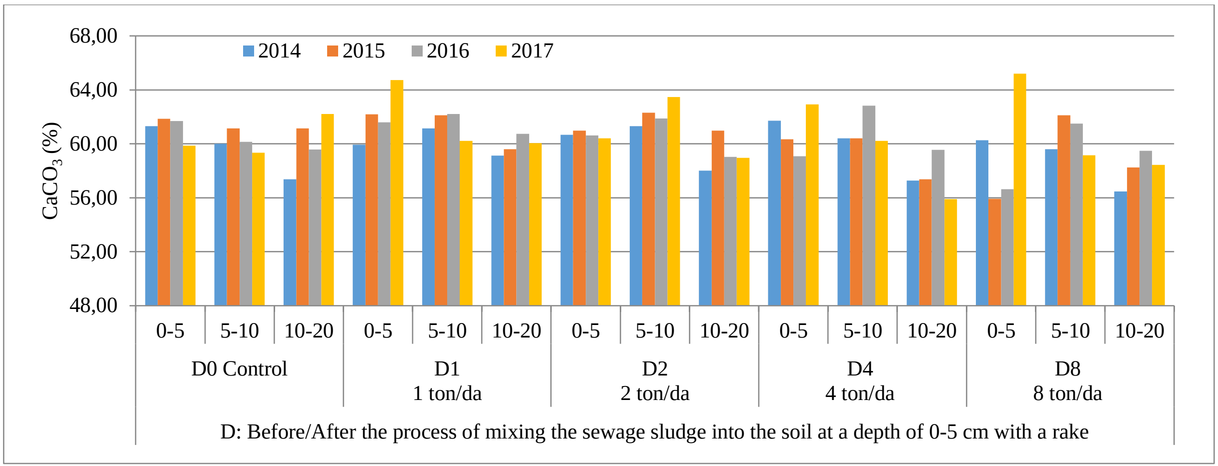


Figure 8. Effect of different doses of sewage sludge applications (D) on the average values of soil $CaCO_3$(%) content

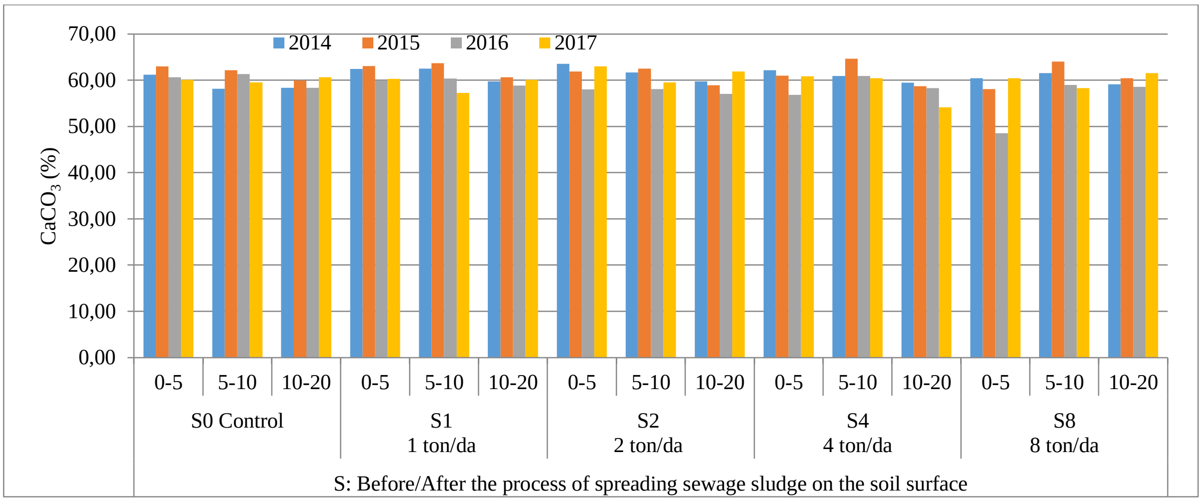


Figure 9. Effect of different doses of sewage sludge applications (S) on the average values of soil $CaCO_3$(%) content

Soil pH: When the pH values of soil samples taken for 3 years after sewage sludge (SS) applications were examined, it was not observed statistically significant difference, when compared to before the first application (2014) (Figure 10,11). Soil pH average values varied between 7.28 - 7.90.

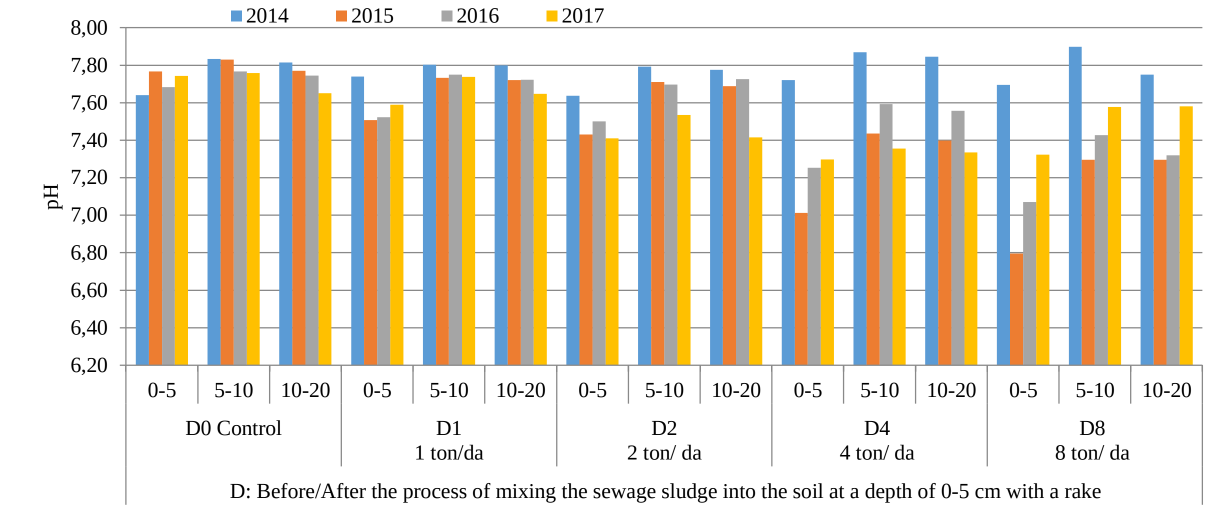


Figure 10. Effect of different doses of sewage sludge applications (D) on the average values of soil pH

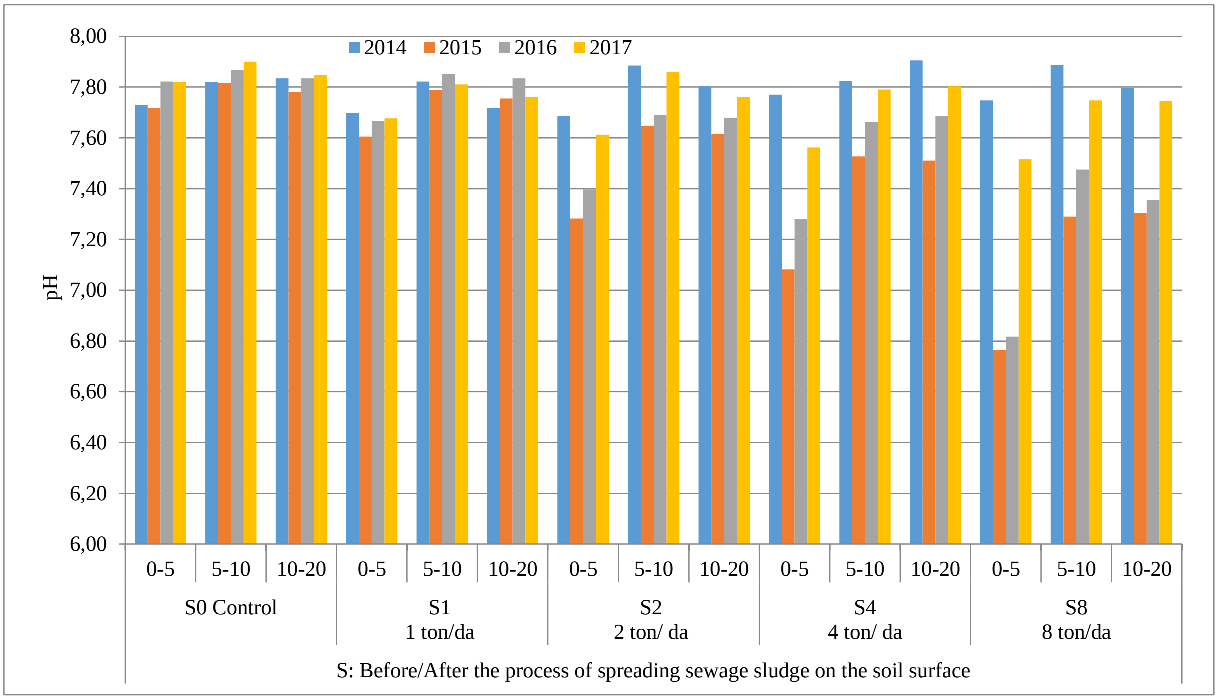


Figure 11. Effect of different doses of sewage sludge applications (S) on the average values of soil pH

Stability Index: If the stability index values obtained in the experiment are examined in detail, the stability index values of the soil samples taken from 0-5 cm depth before SS application (2014) vary between 0.61 and 0.81. This situation is an indication that the aggregate structures are not resistant to erosion. As a result of mixing SS into the soil, stability index values increased in all treatments compared to the control when the 2015 data were analyzed. The highest increase was obtained as 4.66 in 4 tons/da application. The stability index value obtained at 8 tons/da application dose was 4.17. This shows that intensive sewage sludge application is not effective in the formation of durable aggregates above a certain dose. When the stability index values obtained in 2016 and 2017 are analyzed, it is seen that a significant decrease occurred compared to the values obtained in 2015. This situation shows that this application was significantly effective in the first year. but its effectiveness gradually decreased in the following years. When the data of 2015 were analyzed after the application of SS as spreading on the soil surface, an increase was observed in the stability index values in parallel with the amount of dose applied to the soil surface. While this value was determined as 1.41 in the control application, this value was determined as 5.14 in the highest dose application of 8 tons/da. In 2016, this value decreased, and it was determined that durable aggregate structures were observed only in 4 tons/da and 8 tons/da applications. In 2017, it was determined that all of these applications on the soil lost their effect in terms of stability index value. In short, it is seen that SS mixing or spreading applications at a depth of 0-5 cm maintained their effectiveness in terms of stability index value for 1 year and these activities decreased in the following years (Figure 12, 13).

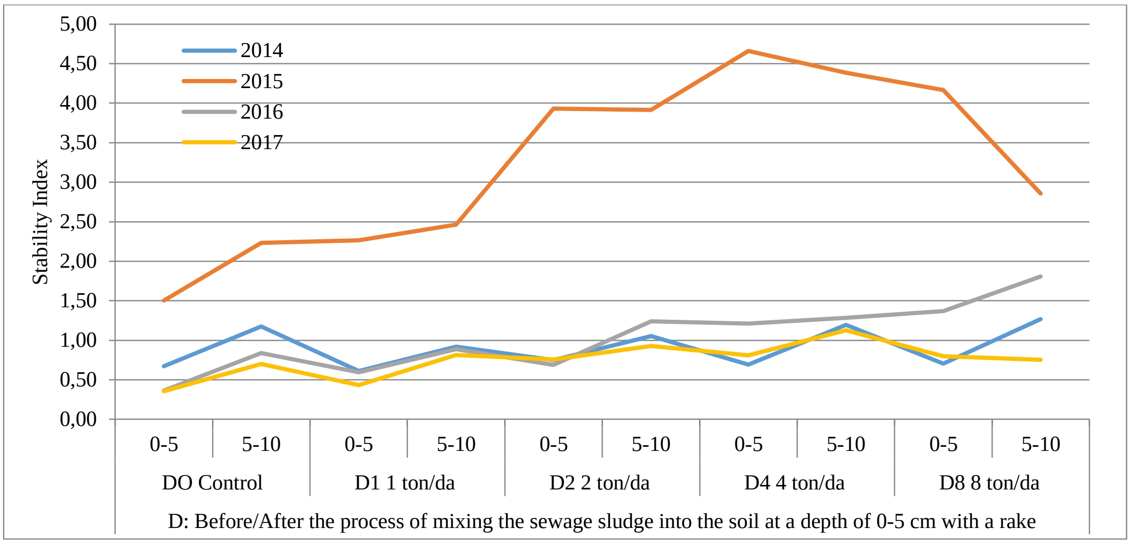


Figure 12. Effect of different doses of sewage sludge applications (D) on the average values of Stability Index

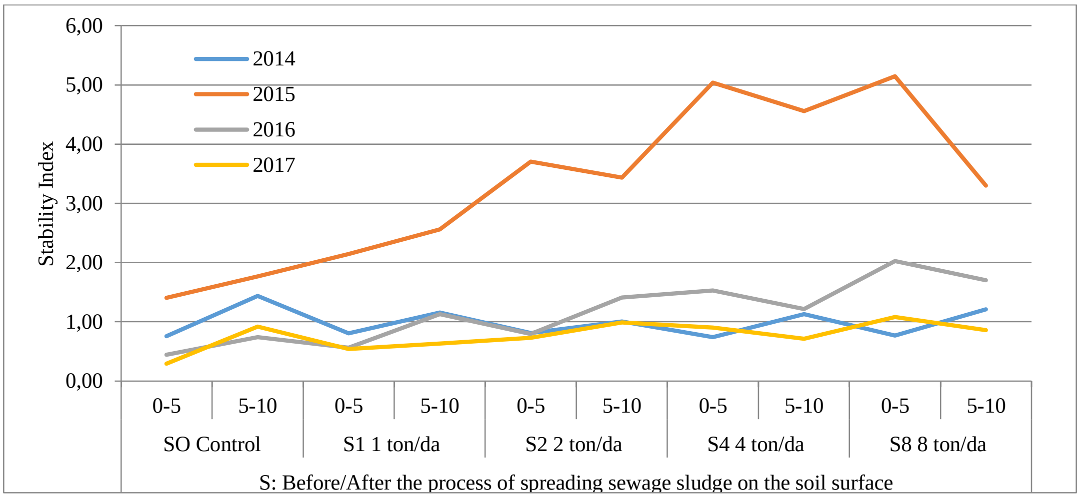


Figure 13. Effect of different doses of sewage sludge applications (S) on the average values of Stability Index

The stability index values determined in the soil samples taken from 5-10 cm depth before SS application (2014) vary between 0.92 and 1.43. Stability index values below 1.5 indicate that these soil samples taken from 5-10 cm depth have aggregate structures that are not resistant to erosion. When the data obtained in 2015 in the treatment of SS applied as soil mixing were examined, stability index values were obtained above 1.5 in all application doses except control. The highest stability index value was 4.39 in 4 tons/da application. In the 8 ton/da application, while an increase was observed compared to the control, the stability index value was less than the value obtained in the 4 ton/da application. SS applied as spreading to the soil was quite effective in terms of stability index in 2015 compared to the control. The highest stability index value was obtained as 4.56 in 4 tons/da application. The value obtained in the 8 tons/da application was close to the 2 tons/da application.

Mechanical Stability: If the mechanical stability values obtained in the study are examined in detail, the mechanical stability values of soil samples taken from 0-5 cm depth before SS application (2014) vary between 43.30 and 51.08%. This is an indication that the resistance of the soils against wind erosion is quite low. After the mixing of SS with the soil with a rake, mechanical stability values increased in all applications compared to the control when the 2015 data were analyzed. The highest increase was obtained as 79% in 8 tons/da application. When the mechanical stability values obtained in 2016 and 2017 are analyzed, it is seen that a significant decrease occurred compared to the values obtained in 2015. This shows that this application was significantly effective in the first year, but its effectiveness gradually decreased in the following years. When the data of 2015 after the application of SS as spreading on the soil surface were analyzed, an increase in mechanical stability values was observed in parallel with the amount of dose applied to the soil surface. While this value was determined as 58.63% in the control application, it was determined as 80.76% in the highest dose application of 8 tons/da. In 2016, this value decreased, and it was determined that aggregate structures resistant to erosion against wind erosion were observed only in 4 tons/da and 8 tons/da applications. In 2017, it is seen that these applications gradually lost their meaning in terms of mechanical stability value (Figure 14, 15).

In short, it was determined that the applications as mixing or spreading of the SS made at a depth of 0-5 cm with a rake to the soil increased and maintained their effectiveness in terms of mechanical stability value for 1 year, but gradually decreased in mechanical stability values in the following years.

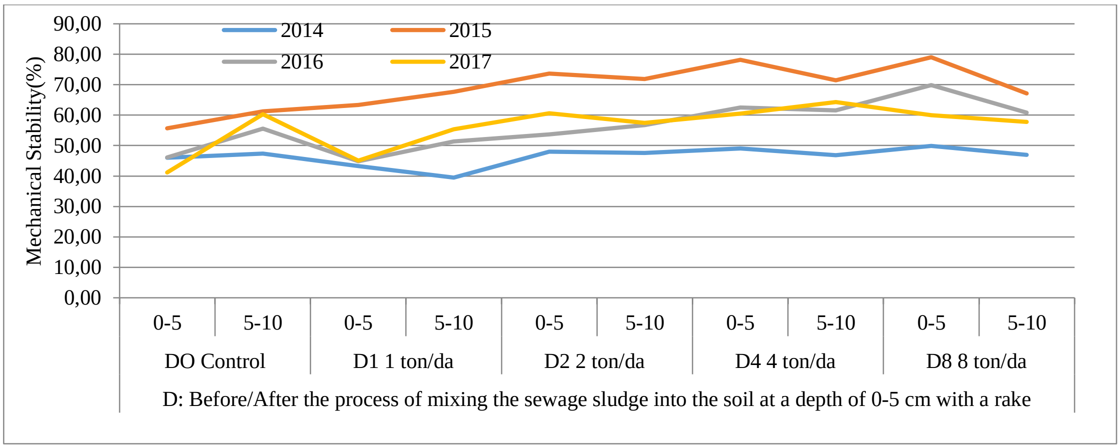


Figure 14. Effect of different doses of sewage sludge applications (D) on the average values of mechanical stability (%)

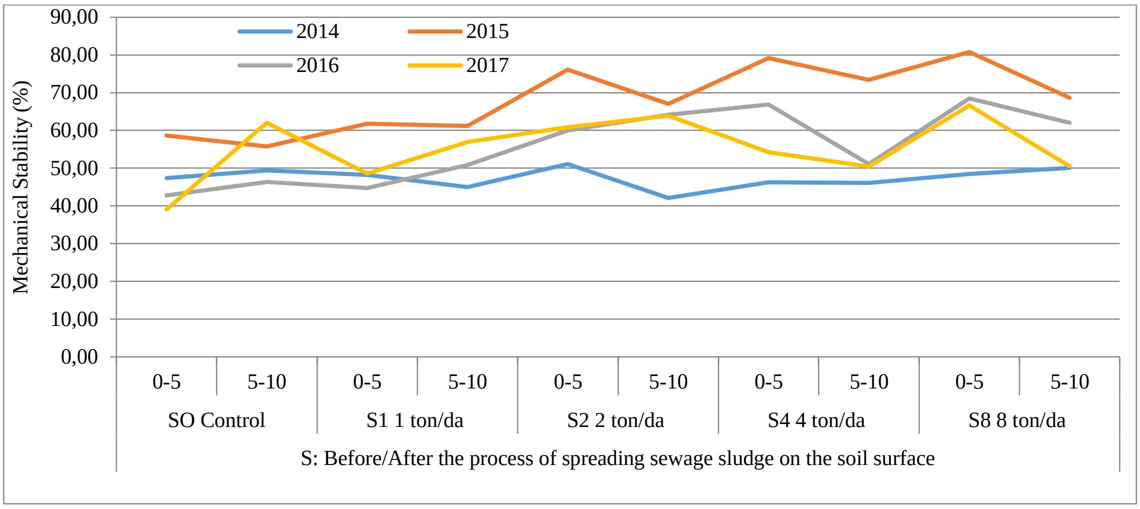


Figure 15. Effect of different doses of sewage sludge applications (S) on the average values of mechanical stability (%)

The mechanical stability values determined in soil samples taken from 5-10 cm depth before the application of sewage sludge (2014) vary between 39.48 and 50.08%. Mechanical stability values below 50% indicate that these soil samples taken from 5-10 cm depth have aggregate structures that are not resistant to erosion. When the mechanical stability values obtained in 2015 in the treatment of sewage sludge applied to the soil by mixing with a rake were examined. mechanical stability values were above 50% in all application doses except control. The highest mechanical stability value was determined as 71.85% and 71.43% at 2 and 4 tons/da of SS applications. respectively. At 8 tons/da of SS application. the mechanical stability value was determined as 67.13%. Treatment SS applied as spreading to the soil was quite effective in terms of mechanical stability in 2015 compared to the control. The highest mechanical stability value was 73.40% at 4 tons/da of SS application. The value obtained at 8 tons/da of SS application was 68.68%.

CONCLUSION

In the study, in eroded poor pasture soils with at least 250 mm of rainfall, the organic matter content at 0-5 cm depth increased from 0.61% before sewage sludge application to 2.22% three years after the application. However, there was no significant difference in other soil depths at the end of three years. This is thought to be due to the decomposition of organic matter in the soil over time. According to the years, EC values increased approximately twice as much at 0-5 cm depth compared to 2014, the first year without application. But EC values were below the limit value of 4 dS/m in the measurement data of all three years. Lime content and pH values did not show any difference between SS applications and years. Considering the erosion parameters, sewage sludge applied to the soils by mixing with rake or spreading was very effective in the formation of resistant aggregates at both soil depths against wind erosion and it is obvious that its effect on soil and water conservation will increase with regular application. In addition, the importance of the application dose was also revealed in this study. At doses above 4 tons/da, it was observed that decreases in durable aggregate formation could be observed. However, when other parameters such as heavy metal enrichment factors examined in this study are taken into consideration, it is concluded that the application dose of sewage sludge can be 1 ton/da once every 3 years by mixing it into the soil at a depth of 0-5 cm. The study is valid for the ecological conditions of the region where the experiment was conducted. This research, which is a pioneering study, can contribute to the literature by making sewage sludge applications in different soil structures.

ACKNOWLEDGMENTS

This project financially supported by TAGEM (Republic of Türkiye Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies) (Project no:TAGEM/TSKAD/14/A13/P05/04).

REFERENCES

Akça, E. 2001. Determination of the Soil Development in Karapınar Erosion Control Station Fallowing Rehabilitation. University of Çukurova, Institute of Natural and Applied Sciences. Doctoral Thesis. Adana. 195 P.

Ayvaz, Z. 2000. Atıksu Arıtma Çamurlarının Değerlendirilmesi. ÇEVKOR Dergisi, 9 (35): 3–12.

Bağcı, Y. 1993. Konya-Karapınar bölgesinin flora ve vejetasyonu. Selçuk Üni. Fen Bilimleri Enstitüsü, Yüksek Lisans Tezi, Konya.

Bilgin, N., H. Eyüpoğlu, H. Üstün. 2002. Biyokatıların (Arıtma Çamurlarının) Arazide Kullanımı. Köy Hizmetleri Genel Müdürlüğü Ankara Araştırma Enstitüsü – ASKİ Arıtma Tesisi Başkanlığı, Ankara.

Birand, H. 1970. Die vermütung der Artemisia steppe bei Karapinar in Zentralanatolien. Vegetatio, 20: 21-47.

Bot, A., J. Benıtes. 2005. The importance of soil organic matter. Key to drought-resistant soil and sustained food and production. In: FAO Soils Bulletin (FAO), no. 80 / FAO, Rome (Italy). Land and Water Development Div. 78 p.

Chepil, W. S. 1962. A Compact rotary sieve and the importance of dry sieving in physical soil analysis. Soil Sci. Soc. Am. Proc. 26:4-6.

Çelebi, H. 1981. Iğdır Devlet Üretme Çiftliği Arazisinde Rüzgâr Erozyonuna İlişkin Araştırmalar, Atatürk Üni. Yay. No: 578, Ziraat Fak. Yay. No: 262 Araştırma Serisi No: 173, Erzurum.

EPA 1994. Land Application of Biosolids, Process Desing Manual. USEPA, Cincinnati Ohio, 12–21.

Groneman, A.F. 1968. The soils of the wind erosion control camp area, Karapınar, Turkey. Ph D. Thesis, Agric Univ. Wageningen, Netherlands, 160.

Hakerler, H. 1980. Kentsel atıkların gübre olarak değerlendirilmeleri. E.Ü.Z.F. Derg., 17:3, 113-131.

İşçi, S. 2006. Arıtma Çamurlarının Özellikleri ve Arazide Değerlendirme İmkanlarının Araştırılması, (Yüksek Lisans Tezi), G.Ü. Fen Bilimleri Enstitüsü Çevre Mühendisliği Ana Bilim Dalı, Ankara.

Kacar, B. 1972. Bitki ve toprağın kimyasal analizleri. Ankara Ü.Z.F. yayınları. 468. A.Ü. Basımevi, Ankara.

Okur, M. 2010. Tarihsel Orta Anadolu Arazi Kullanım Dokusundaki Mera Bitkilerinin Toprak Kalitesine Etkileri. Çukurova Üniversitesi, Fen Bilimleri Enstitüsü Yüksek Lisans Tezi. Adana, 156 S.

Richards, L.A. 1954. Diagnosis and Improvement Saline and Alkaline Soils. U.S. Dep. Agr. Handbook 60.

Şimşekli, N. 2012. Karapınar rüzgâr erozyonunu önleme alanının sürdürülebilir arazi/toprak yönetimi planının geleneksel yapının perspektifinde geliştirilmesi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü Arkeometri Anabilim Dalı, Yüksek Lisans Tezi. Adana.

Tüzüner, A. 1990. Toprak ve Su analizi el kitabı. Köy Hizmetleri Genel Müdürlüğü. Ankara.

ALLEVIATING EFFECTS OF MELATONIN AND JASMONIC ACID IN RICE EXPOSED TO 5-FLUOROURACIL

Armağan KAYA

Alanya Alaaddin Keykubat University, Faculty of Engineering, Fundamental Sciences, Alanya, TURKEY

Corresponding author e-mail: armagan.kaya@alanya.edu.tr

ABSTRACT

Increasing cancer cases in recent years have cause to the use of antineoplastic (anticancer) drugs in high doses and in more combinations. Due to the inadequacy of traditional treatment facilities, antineoplastics can reach the environment from different sources and contaminate different parts of the environment such as soil, groundwater or surface water. 5-Fluorouracil (5-Fu) is a cytotoxic chemotherapy agent widely used in the treatment of various cancers. In this research, some morphological, biochemical and physiological changes in rice plants exposed to 5-Fu were investigated. In addition, the effects of melatonin and jasmonic acid applied to the plants on these changes were determined.

Keywords: 5-Fluorouracil, melatonin, jasmonic acid

INTRODUCTION

Pharmaceutical drugs are compounds used in the prevention, diagnosis and treatment of various diseases in humans and animals. These drugs are biologically active substances and are designed to have specific effects. Pharmaceutical drugs are classified according to their application areas as analgesic/anti-inflammatory drugs, antibiotics, anticonvulsants, heart drugs, cholesterol drugs and chemotherapy drugs, birth control and hormonal drugs and other drugs (stomach, sugar, etc.) (Savcı, 2010; Ustun Odabas, vd, 2020). The primary sources of pharmaceutical drugs in the environment are wastewater from hospitals and other healthcare facilities, domestic wastewater, pharmaceutical industry wastewater, and leaks from storage areas. Since pharmaceutical compounds that enter the environment through various means generally have long half-lives, they can accumulate in groundwater, surface water and soil and harm the plants and animals in the environment (Güler ve Can, 2017; Turhan, 2021; Kaya, 2023).

5-Fluorouracil (5-Fu) is a cytotoxic chemotherapy agent widely used in the treatment of various cancers (Misik ve ark. 2019). This chemotherapy agent is present in both surface water and wastewater. Therefore, it has a strong potential to contaminate agricultural areas. However, there is a huge gap in the literature regarding how this drug affects crop plants.

Plants develop various morphological, physiological and molecular responses to cope with environmental stresses. In addition, exogenous application of some compounds which are naturally synthesized in plants increases the stress tolerance of plants. Melatonin (N-acetyl-5-methoxytryptamine) and jasmonic acid (JA) are two compounds often used to increase stress tolerance of plants exposed to stress conditions. JA is a phytohormone associated with plant growth processes and also plays an important role as a signaling molecule in the formation of plant stress responses. (Wasternack, 2013). Melatonin is a compound that plays a role in the regulation of various physiological processes in both plants and animals and is also an important antioxidant molecule (Surendran et al., 2012; Kaya and Doğanlar, 2019).

In this study, the phytotoxic effects of 5-Fu were investigated using rice (*Oryza sativa*) as a model plant. Additionally, the effects of JA and melatonin on the phytotoxicity of 5-Fu were determined.

MATERIAL AND METHOD

Rice (*Oryza sativa* L.) seedlings were grown from certified seeds of the Luna variety. Rice seeds were sterilized with NaClO and then kept in distilled water for 6 hours. Then, seeds were sown in pots containing peat and perlite (3:1) and the pots were kept in the climate chamber. 8-day-old seedlings were divided into 4 groups and the treatments were done as follows:

Table 1. Experimental design

Groups	Treatments
Control	Distilled water
S	5-Fu (124 ng/L)
S+Mel	5-Fu (124 ng/L) + Foliar Mel (150 μM)
S+JA	5-Fu (124 ng/L) + Foliar JA (45 μM)

Treatments are made every other day for 7 days. After 7 days the plants were harvested. Root length and plant height were determined using fresh plants. The remaining plant samples were frozen at -80 °C for physiological and biochemical analyses.
Chlorophyll and carotenoid contents were determined according to De Kok ve Graham (1980) and Lichtenthaler ve Welburn (1983). MDA content analyzed according to Heath ve Packer (1968). For enzyme analyses, leaves were extracted according to Andrews et al. (2005) and measured activities of ascorbate peroxidase (Cakmak, 1994) and catalase (Luck, 1963).
Statistical analyses were performed using the IBM SPSS 20.0 package program. Differences between the means of different application groups were determined according to the Tukey test.

RESULTS AND DISCUSSION

Root Length and Plant Height

5-Fu decreased root length in rice. However, exogenous Mel and JA applications increased root length. Root length of plants treated with both JA and Mel was found to be higher than the control (Fig 1) ($P<0,05$).
Plant height decreased in plants exposed to 5-Fu. However, Mel and JA applications ameliorated this decrease. JA showed a stronger curative effect on plant height than Mel ($P<0,05$) (Fig. 1)

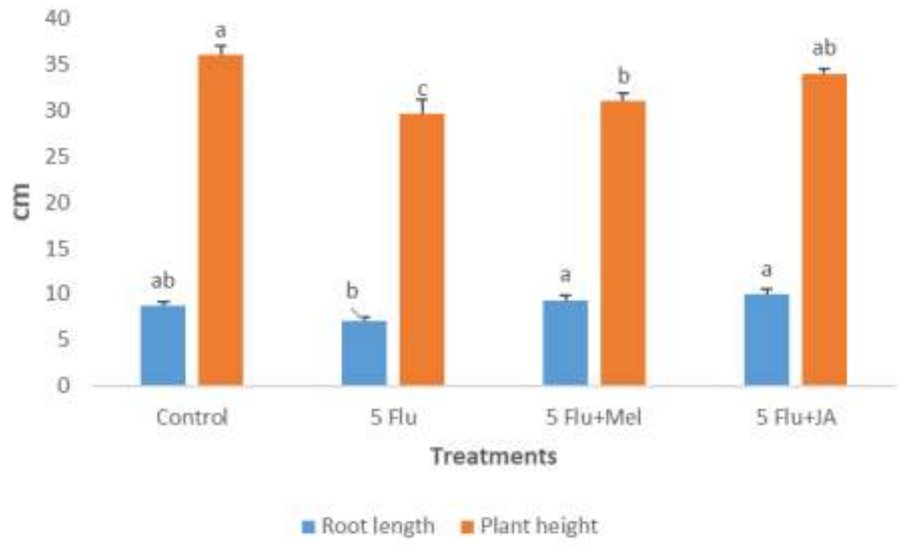


Fig 1. Changes in root length and plant height in plants treated with 5-Fu, Mel and JA.

Chlorophyll and carotenoids Contents
5-Fu application decreased the chlorophyll content in plants. Foliar Mel and JA applications increased the total chlorophyll content back to control level. Similar to chlorophyll content, carotenoid contents decreased as a result of 5-Fu application. However, Mel and JA applications had no significant effect on carotenoid content (*P<0,05*) (Fig 2).

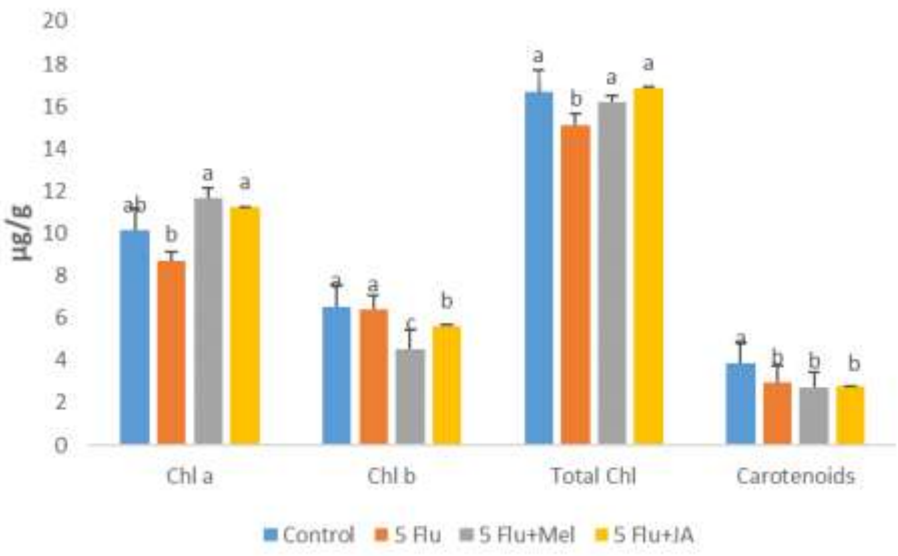


Fig 2. Changes in contents of chlorophyll and carotenoid in plants treated with 5-Fu, Mel and JA

MDA content
5-Fu application significantly increased the MDA content in rice leaves. Exogenous Mel and JA applications reduced the MDA accumulation in plant tissues, but the lowest MDA content was found in the control group (*P<0,05*) (Fig 3).

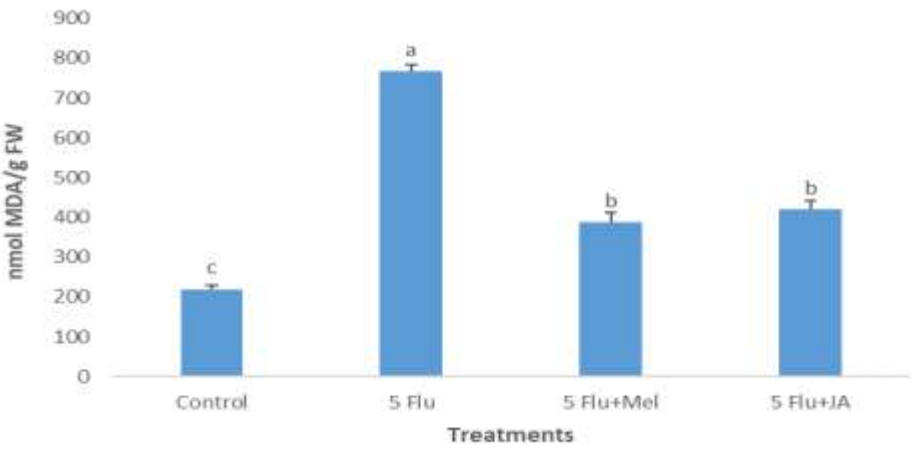


Fig 3. Changes in MDA contents of plants treated with 5-Fu, Mel and JA

Activities of ascorbate peroxidase (APX) and catalase (CAT)

5-Fu application decreased APX activity in all plants compared to control. Foliar JA and Mel applications did not cause any significant change in APX activity of plants exposed to 5-Fu (*P<0,05*) (Fig 4).

CAT activity increased all plants exposed to 5-Fu. Foliar Mel application increased CAT activity, whereas JA application did not cause any significant change in CAT activity (*P<0,05*) (Fig 4).

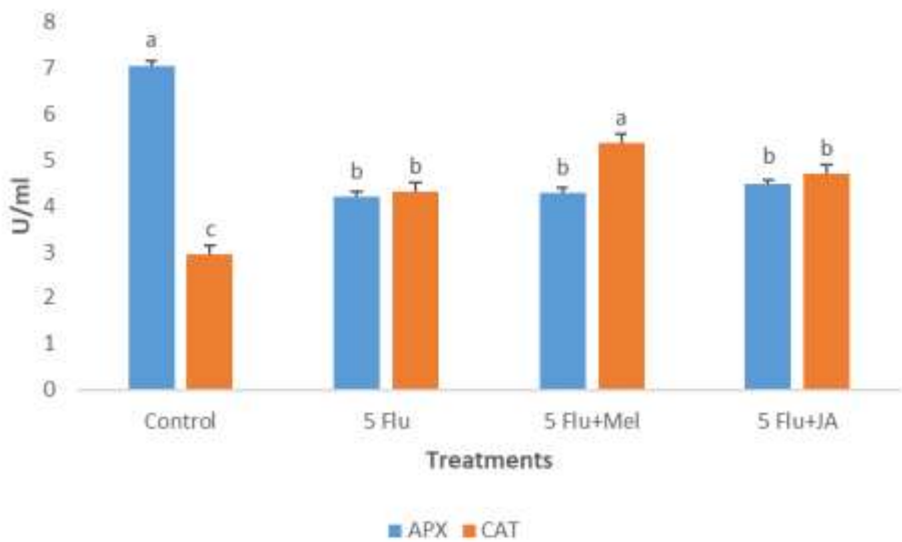


Fig. 4. Changes in activities of ascorbate peroxidase and catalase of plants treated with 5-Fu, Mel and JA

As a result, 5-Fu application decreased plant height, root length, total chlorophyll and carotenoid content and APX activity, and increased CAT activity and MDA content. Additionally, foliar Mel and JA applications alleviated the phytotoxic effects of 5-Fu by increasing plant height, root length, chlorophyll content and decreasing MDA content. However, both compounds did not alter APX activity, while only foliar Mel application increased CAT activity.

REFERENCES

Andrews, C.J., Cummins, I., Skipsey, M., Grundy, N.M., Jepson, I., Townson, J., Edwards, R. 2005. “Purifcation and characterisation of a family of glutathione transferases with roles in herbicide detoxifcation in soybean (Glycine max L.); selective enhancement by herbicides and herbicide safeners”, Pesticide Biochemistry and Physiology, 82(3), 205-219.

Cakmak, I. 1994. “Activity of ascorbate-dependent H2O2-scavenging enzymes and leaf chlorosis are enhanced in magnesium-deficient and potassium deficient leaves, but not in phosphorus-deficient leaves”, Journal of Experimental Botany, 45, 1259–1266.

De-Kok, L., Graham, M. 1980. “Levels of pigments, soluble proteins, amino acids and sulfhydryl compounds in foliar tissue of Arabidopsis thaliana during dark induced and natural senescence”, Plant Physiology and Biochemistry, 27, 133-142.

Güler, Ü.A., Can, Ö.P. 2017. “Kimyasal kontaminantların çevre sağlığı ve gıda güvenliği üzerine etkileri”, Sinop Üniversitesi Fen Bilimleri Dergisi, 170-195.

Heath, R.L., Packer, L. 1968. “Photoperoxidation in isolated chloroplast, I. Kinetics stoichiometry of fatty acid peroxidation”. Archives of Biochemistry and Biophysics, 125, 180–198

Kaya A. (2023) Ascorbate alleviates phytotoxic effects of enrofloxacin in pepper (Capsicum annuum) seedlings. Russian Journal of Plant Physiology. 20,40.

Kaya, A., Doganlar, Z.B. 2019. Melatonin improves the multiple stress tolerance in pepper (Capsicum annuum), Scientia Horticulturae, 256, 108509.

Lichtenthaler, K., Welburn, A.R. 1983. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Botanisches Institutder Univeristat, Kaiserstran βe 12, Postfach 591-592.

Luck, H., 1963. Catalase. In Methods of Enzymatic Analysis, in: Bergmeyer, H.U. (Ed.), Academic Press, New York. pp. 885–888.

Mišík, M., Filipic, M., Nersesyan, A., Kundi, M., Isidori, M., Knasmueller, S. 2019. “Environmental risk assessment of widely used anticancer drugs (5-fluorouracil, cisplatin, etoposide, imatinib mesylate)”, Water Research 164, 114953.

Özhan Turhan D., & Gungordu A. (2022) Developmental, toxicological effects and recovery patterns in Xenopus laevis after exposure to penconazole-based fungicide during the metamorphosis process. Chemosphere, 303 (3), 135302.

Savcı S. (2013) A review of occurrence of pharmaceuticals in sediments. *African Journal of Biotechnology*. 12(29): 4539-4541.

Surendran, D., Geetha, C.S., Mohanan, P.V. 2012. “Amelioration of melatonin on oxidative stress and genotoxic effects induced by cisplatin in vitro”, Toxicology Mechanisms and Methods, 22(8), 631-637.

Ustun Odabasi S, Altın S.H., & Büyükgüngör H. (2020) Sucul Ortamdaki Bazi Mikrokirleticilerin Oluşumu, Durumu ve İleri Oksidasyon Prosesleri ile Giderilmesi. Niğde Ömer Halisdemir Üniversitesi Mühendislik Bilimleri Dergisi. 9(1): 57-71.

EVALUATION ON THE IMPACT OF GLOBAL WARMING ON ENTOMOPATHOGENIC NEMATODES

Alperen Kaan BÜTÜNER[1], İsmail Alper SUSURLUK[1]

[1] Bursa Uludağ University, Faculty of Agriculture, Plant Protection Department, Bursa, Türkiye
Corresponding author e-mail: alperenbutuner@uludag.edu.tr

ABSTRACT

For many years, pesticides have been used in agricultural production areas to control pests. However, restrictions on pesticide use have been increasingly imposed due to the understanding of their toxic effects on non-target organisms in recent years. This has highlighted other methods of pest control. In this context, Entomopathogenic nematodes (EPNs), widely used in biological control, have come to the forefront. Effective utilization of EPNs in agricultural fields depends significantly on environmental factors. Recently, global warming has been notably affecting agricultural areas, particularly causing concerns about drought and high temperatures, which are anticipated to negatively impact EPNs already in use or planned for use. Therefore, developing EPN isolates adapted to our country's conditions, resilient to high temperatures and drought, becomes crucial for future biological pest control strategies in agricultural areas.

Keywords: Biological control, Entomopathogenic Nematodes, Drought, Global warming

INTRODUCTION

There are numerous factors causing yield losses in agricultural production areas. One of the most significant factors contributing to these losses is known as pests. For many years, chemical pesticides have been employed in the control of pests (Baker et al., 2020; Veres et al., 2020; Şahin et al., 2023; Susurluk & Bütüner, 2024; Bütüner et al., 2024a). However, recent studies have revealed the unintended toxic effects of pesticides on non-target organisms. Consequently, the European Union has imposed restrictions on pesticide use in agricultural production areas (Robin & Marchand et al., 2019; Dede et al., 2022; Marchand, 2023; Gensch et al., 2024). This development has brought alternative methods of pest control to the forefront, with biological control emerging as one of the most important and widely used among them (Van Lenteren et al., 2020; Agboka et al., 2024; Bütüner et al., 2024b).

One of the most commonly used organisms in biological control is Entomopathogenic Nematodes (EPNs). EPNs belong to the Secernentea class, Rhabditida order, and families Heterorhabditidae and Steinernematidae. These organisms are known as obligate endoparasites because they require a host organism to complete their life cycle (Boemare et al., 1996; Ehlers, 2001; Koppenhöfer et al., 2007). Generally, their life cycle consists of egg, Juvenile 1, Juvenile 2, Juvenile 3 (Infective Juvenile or IJ), Juvenile 4, and adult stages. These organisms actively seek and infect their hosts only during the Infective Juvenile (IJ) stage (Stock et al., 2002; Lewis et al., 2006; Susurluk & Ehlers, 2008; Bütüner et al., 2023). When IJs encounter their hosts, they enter the host tissue through natural openings (mouth, anus) or pre-existing wounds. Once inside the host tissue, they release gram-negative bacteria belonging to the Enterobacteriaceae family, with which they live in a symbiotic relationship (Dix et al., 1992; Stock, 1998; Shapiro-

Ilan & Gaugler, 2002). These gram-negative bacteria are specialized at the family level in EPNs. Symbiotic bacteria associated with the Heterorhabditidae family are known as *Photorhabdus* spp. whereas those associated with the Steinernematidae family are known as *Xenorhabdus* spp. (Boemare et al., 1996; Ehlers, 2001; Susurluk et al., 2001; Ciche et al., 2006; Susurluk, 2008; Ulu & Susurluk, 2024).

Entomopathogenic nematodes are utilized for potential control of various pests, especially in field conditions and greenhouses such as *Frankliniella occidentalis* (Thysanoptera: Thripidae) (Dlamini et al., 2020; Zhang et al., 2021; Dede et al., 2022; Yüksel et al., 2022; Bütüner et al., 2024b). However, several factors significantly influence their activity on hosts and their survival conditions. Among these factors, temperature and soil moisture (drought conditions) are paramount (Ulu & Susurluk, 2014; Lillis et al., 2022; Bütüner et al., 2023; Ulu & Erdoğan, 2023; Susurluk & Bütüner, 2024). Increases in temperature can enhance the metabolism and infectivity of EPNs, thereby shortening their development periods. Conversely, excessively high temperatures can negatively impact their activity and survival. Similarly, low temperatures can reduce their activity and slow EPN development (Aryal et al., 2022; Bütüner & Susurluk, 2023; Dzięgielewska et al., 2023).

Temperature tolerance varies among EPN species and is often associated with their geographic distribution. Some species thrive in warmer climates, while others are better adapted to cooler regions. This adaptability allows EPNs to achieve greater success both in natural habitats and agricultural environments (Susurluk & Ulu, 2015; Lee et al., 2016; El Khoury et al., 2018; Bütüner & Susurluk, 2023).

In recent years, assessing the potential impact of global warming and consequent droughts on EPNs widely used in biological control in agricultural production areas, both globally and in our country, is of utmost importance (Susurluk & Ulu, 2015; Aryal et al., 2022; Dzięgielewska et al., 2023). Determining the effect of these conditions is believed to pave the way for more effective utilization of EPNs. Additionally, it is thought that developing isolates resistant to high temperatures and drought could enable EPNs to be used more effectively under adverse conditions. This understanding is crucial for optimizing the efficacy of EPNs in agricultural practices amidst changing environmental conditions.

THE EFFECT OF DROUGHT ON EPNs

Entomopathogenic Nematodes exhibit distinct behaviours and capabilities in soils characterized by heavy, clayey texture and compact particle structures. Particularly, it is known that in soils with sandy-loamy texture, EPNs can more easily access their hosts and have greater mobility, whereas such environments severely restrict the host-seeking potential of IJ (Susurluk & Ehlers, 2008; Yadav & Lalramliana, 2012; Ulu & Susurluk, 2014; Susurluk & Ulu, 2015). However, studies have indicated variability in the impact of soil moisture level on different EPN species, hybrid strains, and isolates (Brown & Gaugler, 1997; Radová & Trnková, 2010; Frankenstein et al., 2024). Susurluk & Bütüner (2024) examined the efficacy of *Heterorhabditis bacteriophora* HBH hybrid strain, *Steinernema carpocapsae* TUR-S4, *Steinernema feltiae* TUR-S3, and S-Bilecik isolates on *Tenebrio molitor* (Coleoptera: Tenebrionidae) larvae under different doses and soil moisture conditions. They found that EPNs adapted to local conditions, such as the *H. bacteriophora* HBH hybrid strain, exhibited effective control over hosts even at low soil moisture levels. Soil moisture level not only influences the efficacy of EPNs on hosts but also affects their physiological characteristics, such as reproductive capacity, both positively and negatively. Nevertheless, comprehensive studies regarding the effects of soil moisture on different life stages of EPNs are still lacking (Nouh, 2022; Bütüner & Susurluk, 2023; Susurluk & Bütüner, 2024).

THE EFFECT OF TEMPERATURE ON EPNs

High temperatures are widely recognized as one of the most significant abiotic factors that adversely affect EPNs, notably by significantly shortening their lifespan. While some isolates are known to tolerate high temperatures to a certain extent, studies have identified the duration of exposure to high temperatures as a critical determinant (Kung et al., 1991; Lee et al., 2016; Lillis et al., 2022; Susurluk & Bütüner, 2024). Recent research has also shown that high temperatures can increase the efficacy of certain EPN isolates on their hosts while simultaneously reducing their lifespan. Conversely, very low temperatures have been found to diminish the effectiveness of EPNs on hosts and slow their development (Radová & Trnková, 2010; Nouh, 2022; Devi, 2024). Furthermore, studies have indicated that prolonged exposure to high temperatures negatively affects physiological traits such as reproductive capacity in EPNs. In a study by Bütüner et al. (2023), various EPN isolates were exposed to high temperatures over a prolonged period of three weeks. The findings revealed that increased temperature and duration negatively impacted the efficacy of EPNs on hosts, with a significant portion of the EPNs exposed to high temperatures dying during the exposure period. Similarly, in a study conducted by Susurluk & Bütüner (2024), prolonged exposure of the *H. bacteriophora* HBH hybrid strain to high temperatures resulted in changes in reproductive capacity, hermaphrodite length, and egg size. The results indicated that reproductive capacity and hermaphrodite length were adversely affected with increasing temperature and duration of exposure. These findings underscore the critical importance of temperature management in optimizing the efficacy of EPNs in biological control strategies, highlighting the need for further research into the specific impacts of temperature on different stages and traits of EPNs.

CONCLUSIONS

Entomopathogenic nematodes are recognized for their highly effective use in biological control against pests. However, there are still many aspects of EPNs that remain not fully understood, such as their mechanisms for locating hosts and whether they follow each other while searching for hosts (Erdogan et al., 2021; Trejo-Meléndez et al., 2024). Among these uncertainties, their biological and physiological responses to high temperatures and drought are particularly significant. Research on the effects of high temperatures and drought has often been limited to their efficacy on hosts (Nouh, 2022; Bütüner & Susurluk, 2023; Bütüner et al., 2023; Susurluk & Bütüner, 2024; Matuska-Łyżwa et al., 2024). Studies investigating the physiological traits that may differ in new generations after EPNs infect hosts are relatively scarce. Therefore, studies focusing on adverse conditions, especially concerning EPN species and isolates used in our country, are considered essential (Ulu & Susurluk, 2014; Susurluk & Ulu, 2015). Such research is expected to enhance the effective utilization of EPNs in agricultural production areas, particularly in the face of potential temperature increases and drought due to global warming in the coming years. In this context, active research is being conducted at the Nematology Laboratory, Plant Protection Department, Faculty of Agriculture, Bursa Uludağ University, aimed at developing isolates that are resistant to high temperatures and drought.

REFERENCES

Agboka, K. M., Tonnang, H. E., Abdel-Rahman, E. M., Odindi, J., Mutanga, O., Niassy, S. 2024. Leveraging computational intelligence to identify and map suitable sites for scaling up augmentative biological control of cereal crop pests. Biological Control, 190: 105459.

Aryal, S., Nielsen, U. N., Sumaya, N. H., Wilson, C., Riegler, M. 2022. Effect of temperature on survival of Australian entomopathogenic nematodes and their virulence against the Queensland fruit fly, *Bactrocera tryoni*. BioControl, 67 (6): 617-628.

Baker, B. P., Green, T. A., Loker, A. J. 2020. Biological control and integrated pest management in organic and conventional systems. Biological Control, 140 (1): 104095

Boemare, N., Laumond, C., Mauleon, H. 1996. The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. Biocontrol Science and Technology, 6 (3): 333-346.

Brown, I. M., Gaugler, R. 1997. Temperature and humidity influence emergence and survival of entomopathogenic nematodes. Nematologica, 43 (5): 363-375.

Bütüner, A. K., Ergene, E., İlktan, M., Sepin, S., Susurluk, H., Susurluk, İ. A. 2024b. Impact of some entomopathogenic nematode isolates on the mortality and penetration rate of *Rhyzopertha dominica* and *Tenebrio molitor*. Crop Protection, 179: 106629.

Bütüner, A. K., İlktan, M., Susurluk, A. 2023. Effects of storage temperature on viability and virulence of entomopathogenic nematodes *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* Weiser, 1955 and *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae). Turkish Journal of Entomology, 47 (3): 247-257.

Bütüner, A. K., Şahin, Y. S., Erdinç, A., Erdoğan, H., Lewıs, E. 2024a. Enhancing Pest Detection: Assessing *Tuta absoluta* (Lepidoptera: Gelechiidae) Damage Intensity in Field Images through Advanced Machine Learning. Journal of Agricultural Sciences, 30 (1): 99-107.

Bütüner, A. K., Susurluk, A. 2023. Efficiency of temperature and storage duration on some morphological measurements and reproductive capacity of the entomopathogenic nematode *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)'s Turkish HBH hybrid strain. Turkish Journal of Entomology, 47 (4): 469-476.

Ciche, T. A., Darby, C., Ehlers, R. U., Forst, S., Goodrich-Blair, H. 2006. Dangerous liaisons: the symbiosis of entomopathogenic nematodes and bacteria. Biological Control, 38 (1): 22-46.

Dede, E., Bütüner, A. K., Susurluk, A. 2022. Biocontrol potential of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hybrid strain against the beet webworm, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae). Turkish Journal of Entomology, 46 (4): 399-405.

Devi, G. 2024. Influence of Abiotic Factors on Efficacy of Entomopathogenic Nematodes. International Journal of Plant & Soil Science, 36 (3): 283-290.

Dix, I., Burnell, A. M., Griffin, C. T., Joyce, S. A., Nugent, M. J., Downes, M. J., 1992. The identification of biological species in the genus *Heterorhabditis* (Nematoda: Heterorhabditidae) by cross-breeding second-generation amphimictic adults. Parasitology, 104 (3): 509-518.

Dlamini, T. M., Allsopp, E., Malan, A. P., 2020. Application of *Steinernema yirgalemense* to control *Frankliniella occidentalis* (Thysanoptera: Thripidae) on blueberries. Crop Protection, 128: 105016.

Dzięgielewska, M., Kaczmarek, K., Kruk, K. 2023. The influence of temperature on the biological activity of selected nematode species (Steinernematidae and Heterorhabditidae) under the conditions of their coexistence. Plant Protection Science, 59 (2):

Ehlers, R. U., 2001. Mass production of entomopathogenic nematodes for plant protection. Applied Microbiology and Biotechnology, 56 (5-6): 623-633.

El Khoury, Y, Oreste, M., Noujeim, E., Nemer, N., Tarasco, E. 2018. Effect of temperature on the pathogenicity of Mediterranean native entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from natural ecosystems. Redia, 101: 123-127.

Erdogan, H., Cruzado-Gutierrez, K., Stevens, G., Shapiro-Ilan, D., Kaplan, F., Alborn, H., Lewis, E., 2021. Nematodes follow a leader. Frontiers in Ecology and Evolution, 9: 740351.

Frankenstein, D., Luu, M. S., Luna-Ayala, J., Willett, D. S., Filgueiras, C. S. 2024. Soil moisture conditions alter behavior of entomopathogenic nematodes. Journal of the Science of Food and Agriculture, 104 (7): 4383 – 4390.

Gensch, L., Jantke, K., Rasche, L., Schneider, U. A. 2024. Pesticide risk assessment in European agriculture: Distribution patterns, ban-substitution effects and regulatory implications. Environmental Pollution, 348: 123836.

Koppenhöfer, A. M., Grewal, P. S., Fuzy, E. M., 2007. Differences in penetration routes and establishment rates of four entomopathogenic nematode species into four white grub species. Journal of Invertebrate Pathology, 94 (3): 184-195.

Kung, S. P., Gaugler, R., Kaya, H. K. 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. Journal of invertebrate pathology, 57 (2): 242-249.

Lee, J. H., Dillman, A. R., Hallem, E. A. 2016. Temperature-dependent changes in the host-seeking behaviors of parasitic nematodes. BMC biology, 14: 1-17.

Lewis, E. E., Campbell, J., Griffin, C., Kaya, H., Peters, A., 2006. Behavioral ecology of entomopathogenic nematodes. Biological Control, 38 (1): 66-79.

Lillis, P. E., Griffin, C. T., Carolan, J. C., 2022. The effect of temperature conditioning (9 C and 20 C) on the proteome of entomopathogenic nematode infective juveniles. PloS one, 17 (4): e0266164.

Marchand, P. A., 2023. EU Chemical Plant Protection Products in 2023: Current State and Perspectives. Agrochemicals, 2 (1): 106-117

Matuska-Łyżwa, J., Duda, S., Nowak, D., Kaca, W., 2024. Impact of Abiotic and Biotic Environmental Conditions on the Development and Infectivity of Entomopathogenic Nematodes in Agricultural Soils. Insects, 15 (6): 421.

Nouh, G. M., 2022. Effect of temperature and soil moisture on the efficacy of indigenous and imported strains of the entomopathogenic nematode, *Heterorhabditis* sp. against the black cutworm, *Agrotis ipsilon* (Hufnagel)(Lepidoptera/Noctuidae). Egyptian Journal of Biological Pest Control, 32 (1): 28.

Radová, Š., Trnková, Z., 2010. Effect of soil temperature and moisture on the pathogenicity of two species of entomopathogenic nematodes (Rhabditida: Steinernematidae). Journal of Agrobiology, 27 (1): 1–7

Robin, D. C., Marchand, P. A., 2019. Evolution of the biocontrol active substances in the framework of the European Pesticide Regulation (EC) No. 1107/2009. Pest Management Science, 75 (4): 950-958

Şahin, Y. S., Erdinç, A., Bütüner, A. K., Erdoğan, H., 2023. Detection of *Tuta absoluta* larvae and their damages in tomatoes with deep learning-based algorithm. International Journal of Next-Generation Computing, 14 (3): 555-565.

Shapiro-Ilan, D. I., Gaugler, R., 2002. Production technology for entomopathogenic nematodes and their bacterial symbionts. Journal of Industrial Microbiology and Biotechnology, 28 (3): 137-146.

Stock, S. P., 1998. *Steinernema siamkayai* n. sp. (Rhabditida: Steinernematidae), an entomopathogenic nematode from Thailand. Systematic Parasitology, 41 (2): 105-113.

Stock, S. P., Griffin, C. T., Burnell, A. M., 2002. Morphological characterisation of three isolates of *Heterorhabditis* Poinar, 1976 from the Irish group'(Nematoda: Rhabditida:

Heterorhabditidae) and additional evidence supporting their recognition as a distinct species, *H. downesi* n. sp. Systematic Parasitology, 51 (2): 95-106.

Susurluk, A., 2008. Potential of the entomopathogenic nematodes *Steinernema feltiae*, *S. weiseri* and *Heterorhabditis bacteriophora* for the biological control of the sugar beet weevil *Bothynoderes punctiventris* (Coleoptera: Curculionidae). Journal of pest science, 81: 221-225.

Susurluk, A., Bütüner, A. K., 2024. The role of drought in the efficacy of some entomopathogenic nematodes. Turkish Journal of Entomology, 48 (1): 103-110.

Susurluk, A., Dix, I., Stackebrandt, E., Strauch, O., Wyss, U., Ehlers, R. U. 2001. Identification and ecological characterisation of three entomopathogenic nematode-bacterium complexes from Turkey. Nematology, 3 (8): 833-841.

Susurluk, I. A., Ehlers, R. U., 2008. Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. BioControl, 53 (4): 627-641.

Susurluk, I. A., Ulu, T. C., 2015. Virulence comparisons of high-temperature-adapted *Heterorhabditis bacteriophora*, *Steinernema feltiae* and *S. carpocapsae*. Helminthologia, 52 (2): 118-122.

Trejo-Meléndez, V. J., Ibarra-Rendón, J., Contreras-Garduño, J., 2024. The evolution of entomopathogeny in nematodes. Ecology and Evolution, 14 (2): e10966.

Ulu, T. C., Erdoğan, H., 2023. Field application of encapsulated entomopathogenic nematodes using a precision planter. Biological Control, 182: 105240.

Ulu, T. C., Susurluk, I. A., 2014. Heat and desiccation tolerances of *Heterorhabditis bacteriophora* strains and relationships between their tolerances and some bioecological characteristics. Invertebrate Survival Journal, 11 (1): 4-10.

Ulu, T. C., Susurluk, I. A., 2024. In vitro liquid culture production and post-production pathogenicity of the hybrid *Heterorhabditis bacteriophora* HBH strain. Crop Protection, 175 (1): 106443.

Van Lenteren, J. C., Alomar, O., Ravensberg, W. J., Urbaneja, A., 2020. Biological control agents for control of pests in greenhouses. Integrated pest and disease management in greenhouse crops, 409-439.

Veres, A., Wyckhuys, G. A. K., Kiss, J., Tóth, F., Burgio, G., Pons, X., Avilla, C., Vidal, S., Razinger, J., Bazok, R., Matyjaszczyk, E., Milosavljević, I., Vi Le, X., Zhou, W., Zhu, R. Z., Tarno, H., Hadi, B., Lundgren, J., Bonmatin, M. J., van Lexmond, B. M., Aebi, A., Rauf, A., Furlan, L., 2020. An update of the worldwide integrated assessment (WIA) on systemic pesticides. Part 4: Alternatives in major cropping systems. Environmental Science and Pollution Research, 27 (24): 29867-29899

Yadav, A. K., Lalramliana., 2012. Soil moisture effects on the activity of three entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) isolated from Meghalaya, India. Journal of Parasitic Diseases, 36, 94-98.

Yüksel, E., Imren, M., Özdemir, E., Bozbuğa, R., Canhilal, R., 2022. Insecticidal effect of entomopathogenic nematodes and the cell-free supernatants from their symbiotic bacteria against different larval instars of *Agrotis segetum* (Denis & Schiffermüller)(Lepidoptera: Noctuidae). Egyptian Journal of Biological Pest Control, 32 (1): 54.

Zhang, X., Wu, S., Reitz, S. R., Gao, Y., 2021. Simultaneous application of entomopathogenic Beauveria bassiana granules and predatory mites *Stratiolaelaps scimitus* for control of western flower thrips, *Frankliniella occidentalis*. Journal of Pest Science, 94 (1): 119-127.

FERTILIZATION WITH MINERAL NITROGEN (AMMONIUM NITRATE) IMPROVES GROWTH, LEAVES BIOMASS PRODUCTION AND THEIR BIOCHEMICAL VALUES IN MORINGA OLEIFERA KNOWN AS MIRACILOUS TREE

Afef Hajaji Nasraoui[1], Houda Maaroufi-Dguimi[2], Mejda Abassi[1], Youssef Ammari[1]*

[1]Forest Ecology Laboratory, National Research Institute in Rural Engineering, Water and Forestry, Bp 10, 2080, Ariana, University of Carthage, Tunisia
[2]Department of Biology, Faculty of Science, Al Baha University, P.O.Box 1988. Saudi Arabia

Corresponding author e-mail: hajajiafef@yahoo.fr

ABSTRACT

This study aims to prove that growth and biochemical quality of *Moringa oleifera* the miraculous tree able to grow under different culture conditions, were optimized by nitrogen fertilization of soil. In our case, three-aged seedlings were grown under control condition (without fertiliser) or in presence of NH_4NO_3 as fertilizer. Leaves and flowers numbers, stem diameter and height, relative growth rate (RGR), net assimilation rate (NAR), relative leaf area expansion rate (RGRA) and relative growth rate of the principal stem in height (RGRh) were enhanced by nitrogen fertilizer presence. The high growth level seemed to be the result of increase of chlorophyll a fluorescence parameters principally the maximum quantum efficiency of PSII photochemistry (Fv/Fm). More that, when Moringa reached the most pronounced degree of growth (August, 2023), biochemical measures were realized. At that time of phonologic cycle in which they were generated, a rise in the contents of total phenolic compounds, total flavonoids, condensed tannins, soluble sugars, proline and proteins was observed. Compared to in control trees, dramatic tenor's rise of all secondary metabolites, osmolytes and proteins was shown in fertilezed plants. Recording to our goals, data demonstrated the importance of soil fertilization in Moringa improvement of bioactive compounds richness improving there healthy benefits.

Keywords: Bioactive compounds, Nitrogen fertilizer, Fluorescence, *Moringa oleifera,*

INTRODUCTION

The constraint of low soil fertility in soil is largely associated with welp productivity of crop. For this reason, many manure of fertilization (compost, crop residue…) were applied as optimization approach in order to maximize crop productivity and support species to increase biomass production, especially, under the climatic changes. Several studies (Nalivata et al., 2022; Ouattara et al., 2022; Warttman et al., 2022; Kayouki et al., 2022) prove that small or no fertilization due to financial or socio-economic factors.

Moringa oleifera is widely esteemed for its therapeutic characteristics and nutritional advantages (Padayachee et al., 2020). Hence, this plant has great economic significance. Several efforts have been made to promote its culture extensively in various regions around the globe. However, Moringa culture encounters some challenges as salinity and drought (Sadak et al., 2020; Azeem et al., 2023). So, fertilization was used to enhance plant growth and propagation. Different types of fertilizers were used to enhance plant growth (Ndagi et al., 2023). Inorganic fertilizers increased growth of Moringa and enhance leaf yield (Ngwenya et al, 2022). Recently, Ndagi et al. 2023 showed that organic fertilizer (poultry dropping) gave the best result for Moringa farming. In other environmental conditions, Cobalt and organic fertilization made a promising strategy to improve moringa plant productivity (Gad et al., 2019). Despite the importance of these results, additional investigation is required to determine the effect of these fertilizers on the biochemical properties of this plant, from which its commercial value is derived. The phytocomplex found in Moringa leaves has been extensively linked to a range of advantageous effects on human health such as antioxidant (Sreelatha et al., 2019). Several studies showed that abiotic stress induced antioxidant metabolites and potential in Moringa oleifera (Azeem et al., 2023; Zanella et al., 2019). Studies showed too, that the administration of inorganic fertilizer increased the accumulation of secondary metabolites (Mutua et al, 2021). Conversely, nitrogen fertilization reduced secondary metabolites in *Rosmarinus officinalis* (Bustamante et al., 2020).

In light of Moringa's economic significance, research efforts should be concentrated on enhancing its growth and mitigating environmental stresses, all the while monitoring its active constituents to safeguard its medicinal properties. Hence, the purpose of this study was to determine the effect of ammonium nitrate fertilizer on growth and biochemical components of *Moringa oleifera* seedlings.

MATERIAL AND METHOD

Experimental site description and growth conditions. The experiment was carried out at the research area of National Institute of Research in Rural Engineering, Waters and Forests (INRGREF) of Tunis (Tunisia). Three years-aged *Moringa oleifera* trees previously planted under Tunisian climate conditions (Table 1) were used to realize this study. In November 2022, plants showing a total leaf fall were transferred in pots containing 10Kg of soil to grow under control condition (without fertiliser) or in presence of NH_4NO_3 (250g/pot) as nitrogen fertilizer. The experiment was conducted for 13 months (November, 2022 to December, 2023) for four replicates.

Table 1. Different temperature and rainfall levels during experimental period

	November 2022	June 2023	July 2023	August 2023	Sptember 2023	October 2023	November 2023	December 2023
Temperature (°C)	18,3	25,6	32,5	28,4	27,4	24,9	19,9	15,5
Rainfall	8,3	5,3	0	9	1	0	5,7	6,3

Morphological and growth parameters. Growth and morphological parameters measures have been taken at the first time at November, 2022 (t_0), and later every 30 days during 07 months (June-December, 2023). Leaves and flowers numbers, the principal stem height and diameter were determined at the different times in plants grown in absence of fertilizer (control) or in presence of nitrogen fertilizer (NH_4NO_3).

At August month (2023), total leaves fresh and dry weights were determined. Also, a planimeter (model CI-202, USA) had been used to measure the several leaves parameters (area, length, width, and perimeter).

Relative growth rate (RGR mg. g^{-1}. d^{-1}), net assimilation rate, (NAR g. m^{-2}. d^{-1}), relative leaf area expansion rate, (RGRA mm^{-2}.d^{-1}) and relative growth rate of the principal stem in height (RGRh, cm. cm^{-1}. d^{-1}) were determined according to the following equations respectively

$(lnw_9-lnw_0)/(t_9-t_0);(w_9-w_0)/dt*(lnA_9-lnA_0)/(A_9-A_0);(lnLA_9-lnLA_0)/(t_9-t_0)$ and (lnh_9-lnh_0/t_9-t_0)

W (shoot weight); A (leaf area); DW (increment in leaves biomass); LA (total plant leaves area); h (stem height); t_0 is the time at the beginning (November, 2022) and t_9 at August month (2023) when all growth parameters showed the major values.

Fluorescence measurement and photosynthetic pigments determination. The measurement of fluorescence is carried out on plants previously adapted to darkness for 30min. Following the application of a pulse of saturating light, the fluorescence increases from the ground state Fo (All reaction centers are open) towards a maximum level F_m (all reaction canters are closed). In this case plastoquinone QA, the first electron acceptor emitted by PSII, is completely reduced. This situation allows us to determine the maximum efficiency of PSII in converting light energy into chemical energy. Fluorescence measurements at the beginning (November, 2022) and at August month (2023) were measured by modulated chlorophyll fluorometer (OS1p- Opti-Sciences, USA).

Contents of Chla, Chl tot and carotenoids determination from extracted leaves samples were determined spectrophotometricaly with PG instrument (T60 UV-V /UK) according to Arnon et al. (1956).

Determination of proline and soluble sugars contents. Leaves samples collected at the beginning (November 2022) and at August 2023 were extracted in order to determine proline contents in accordance with Bates (1973) method. Results were expressed as µmol. g^{-1} DW. Dubois et al. (1956) developed method was adopted to determine total soluble sugars, which was defined as µmol. g^{-1} DW.

Proteins and nitrogen crude determination. As described by Pearson (1976), proteins contents of leaves samples collected at the beginning (November 2022) and at August 2023, were determined. Also, according to the equations below, the nitrogen amount and crude protein were respectively estimated:

$$N\ \% = [(V \text{ x } n \text{ x } 0.014)/M] \times 100$$

$$\text{Crude protein } \% = \%\ N \times 6.25. \text{ (N: Nitrogen).}$$

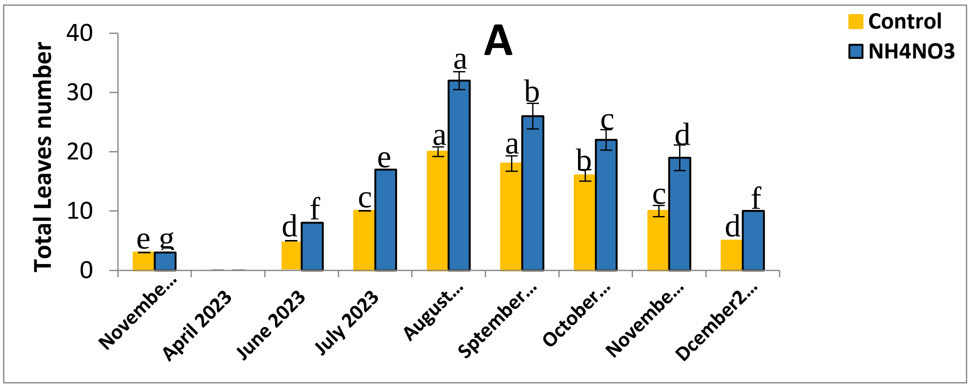
(V: volume of hydrochloric acid used in the titration. n: normality of hydrochloric acid and M: mass of the sample in grams).

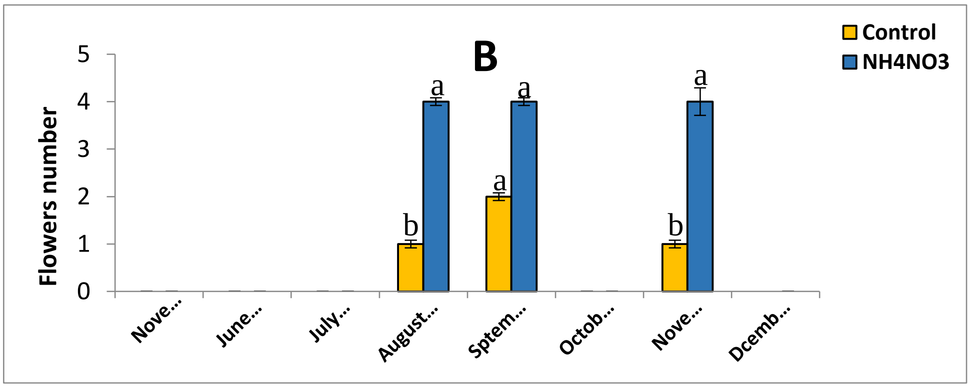
Total phenolic compounds, total flavonoids and condensed tannins contents quantification. To do, leaves samples collected at the beginning (November 2022) and at August 2023 were used. Total phenolic compounds were quantified in accordance with the Folin–Ciocalteu method of Li et al., (2004). To calculate the total phenolic compounds, Gallic acid was used (mg GAE.g^{-1}). Dehpeur et al. (2009) method was adopted to carried out total flavonoid content. Which is expressed in mg quercetin equivalent per gram of dry weight (mg Qu. g^{-1}). To estimate total condensed tannins Ba et al. (2010) method was followed. Total condensed tannins Contents of total condensed tannins were defined in mg catechol equivalent/g dry weight (mg cat. g^{-1}).

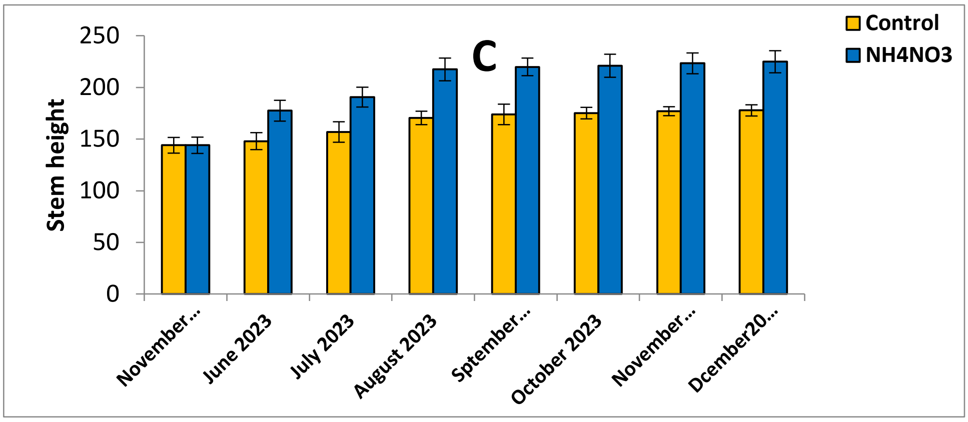
Statistical analysis. Data are means of six replicates. All data were statistically analysed and subjected to a one-way ANOVA analysis based on Tukey's HSD test (IBM SPSS Statistics 23). Correlation was designed by Pearson test at probability levels of 0.01 and 0.05. Letter used to indicate significant differences between treatment means.

RESULTS AND DISCUSSION

Extensive distribution of *Moringa oleifera L.* occurs across the globe areas. It is utilized globally as a multipurpose herbal plant for human consumption and as an alternative medicinal plant. This tree's foliage is rich in antioxidants, including flavonoids, carotenoids, phenolics, and ascorbic acid (Vongsak et al., 2014). Due to its economic importance, numerous studies have been devoted to enhancing the medicinal properties and agricultural practices of this plant (Razis et al., 2014). Our data showed that, at August 2023, M. *oleifera* trees acquired the highest leaf number in both control and fertilized plants (Figure 1A). The leaf number increase is preceded by a temperature peak (28,4°C) dtected in July 2023 (Table1). The increment in leaves number is more significant in fertilized plants (32 leaves) compared to control plants (20 leaves). More that and as shown by Figure 1B, floral blossoming reached a maximum of 4 flowers at August 2023 in fertilized plants. Whereas, in control plants, the maximum of flowers (2) is observed at September 2023 month. After failure and ablotion of these flowers news one in order of 1 and 4 in control and fertilzed trees respecteively, were observed. In fact, it is widely known that the Moringa tree is a miraculous species that grows in several environmental and soil conditions (Karin et al., 2020), but we cannot ignorate the effects of climate change crossing the world and which is resulting in nutriments poor soil. This is why the reconstitution of soils through fertilization appears fundamental to increase soil fertility on the one hand and increase crop yields on the other (Mason et al., 2015; Ouattara, 2017). Accordingly, we can understand the morphological and growth responses of Moringa trees grown on medium added with fertilizer. In addition, results showed that soil nitrogen fertilization improved shoot part growth through improvement of stem growth (Figure 1C, D). In another way, total leaves weights, area, lengh and perimeter were increased when soil was enriched by ammonia nitrate (Table 2).







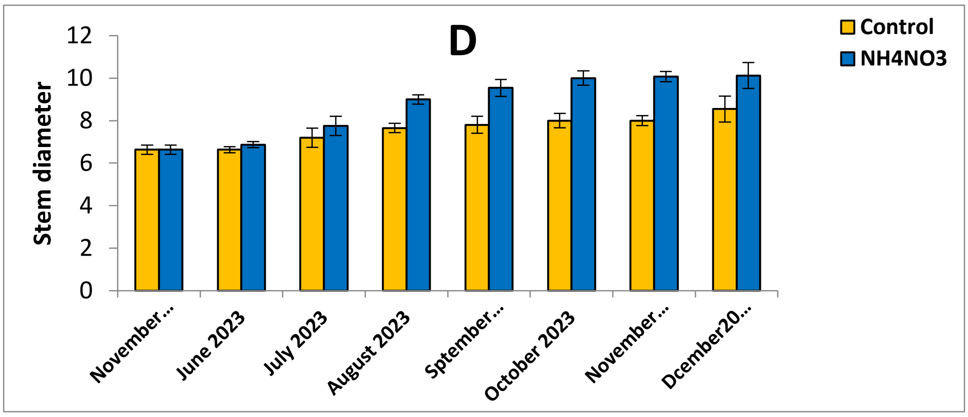


Figure 1. Variation of leaves number (A), flowers number (B), stem height (C) and stem diameter (D) in *Moringa oleifera* grown on different nutritive media (control and NH_4NO_3 fertilisation).

Table 2. Leaves morphological parameters in *Moringa oleifera* grown under different nutritive media (control and NH_4NO_3 fertilisation). (t0: time zero; tf: final time)

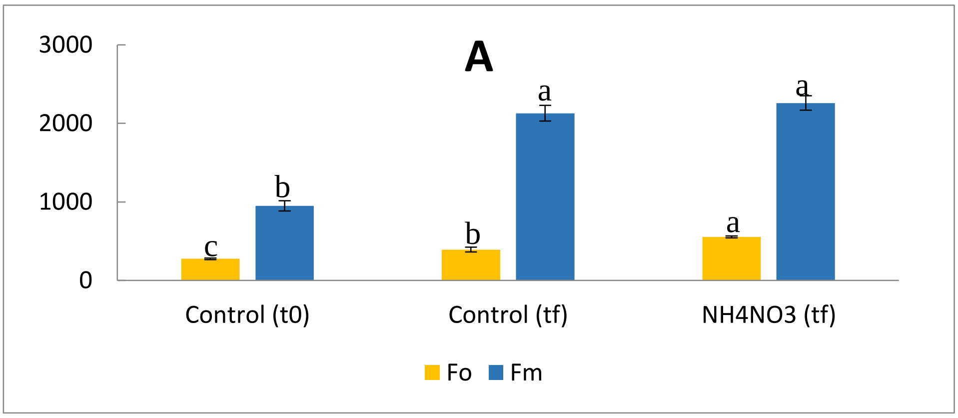
	Total leaves fresh weight (g)	Total leaves dry weight (g)	Total leaves area (cm^2)	Total leaves length (cm)	Total leaves width (cm)	Total leaves perimeter (cm)
Control (t0)	4,2347±0.142^c	0,445±0.022^c	267,94±2.013^c	66,11±4.092^c	28,68±1.28^c	671,36±30.301^c
Control (tf)	16,402±1.01^b	1,286±0.084^b	651,449±22.22^b	132,29±8.086^b	97.609±60.607^b	1438,336±30.05^b
NH_4NO_3 (tf)	38,635±2.35^a	4,117±0.233^a	1171,315±10.102^a	258,27±6.631^a	112,65±9.974^a	2884,32±10.414^a

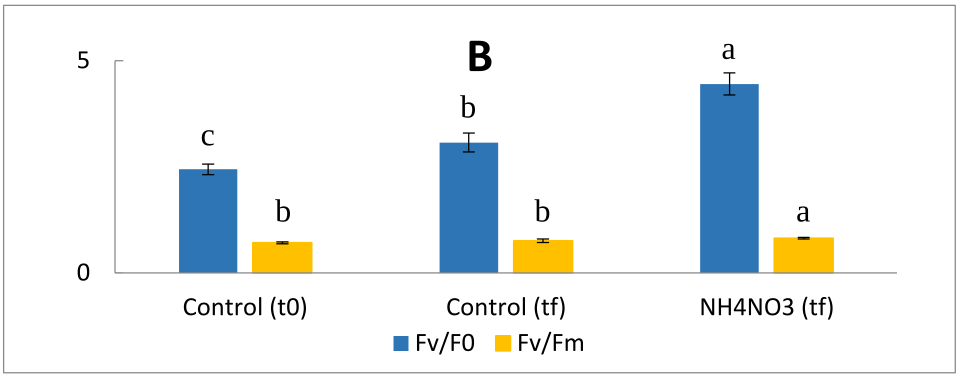
More, relative growth rate (RGR) relative leaf area expansion rate (RGRA) and net assimilation rate (NAR), were remarkably improved in fertilezed Moringa plants (Table 3).

Table 3: Variation of relative growth rate (RGR), relative leaf area expansion rate (RGRA), relative growth rate of principal stem (RGRh) and net assimilation rate (NAR) in *Moringa oleifera* under different nutritive media (control and NH_4NO_3 fertilisation).

Growth parameters	Relative growth rate: **RGR** ($mg.g^{-1}.d^{-1}$)	Relative leaf area expansion rate **RGRA** ($mm^{-2}.d^{-1}$)	Net assimilation Rate **NAR** ($g.m^{-2}.d^{-1}$)	Relative growth rate of principal stem in height **RGRh** ($cm.cm^{-1}.d^{-1}$)
Control	$3.428078\ .10^{-3}$	$2.249205.10^{-3}$	1.314698748	$0.516778.10^{-3}$
NH_4NO_3	$5.597079.10^{-3}$	$3.734479.10^{-3}$	5.056261034	$1.120942.10^{-3}$

Our data were opposit to Ndagi et al, (2023) suggestions, which reported that inorganic fertilizer (NPK) had no significant effect on leaf number and leaf area in *Moringa oleifera*. Whereas, many other resaerchers (Zhang et al., 2019; Amin, 2020; Ngwenya et al., 2022, Phinehas et al., 2022; Hafeez et al., 2023; Huang et al., 2024) have confirmed that inorganic fertilizers application enhanced leaves yield in sevral species such as Camelia, Moringa chick pea, pepper, tomato... Khalaj and Noroozisharaf (2020) proved that an appropriate ammonium nitrate ratio as fertilezer improved plant production in vegetative and in reproductive stages by increasing flowers number. However, ammonia nitrate had a key role in nutrient uptake, plant growth and fruit quality (Pedersen et al, 2019; Yan et al, 2019; Zhang et al, 2019). More precisely work specifed that optimal plant yields and fruit quality were dependent to N fertilization rates (Souza et al., 2020). Also, primary (stem height) and secondary growth (stem diameter) also than stem relative growth in height were rised in Moringa plants grown on added soil by ammonia nitrate. Logically and as described by Jianbo et al. (2020), the first effect of nitrogen fertilization is associated to change of soil properties through cycle of soil nutrient and avilability of nutrients. More that, according to many other researchers (Liu et al., 2013; Kitonyo et al., 2018; Hafeez et al., 2023), nitrogen fertilizer rise significantly soil water content, soil water retention capacity and soil aggregate components amounts. More, at plant level, fertilizer affects its physiology through stimulation of photosynthesis apparatus based on enhacement of photosynthetic pigments (Chlorophylls) accumulation. According to theses ideas, our results (Figure 2A and B) demonstrated an increase of both maximum primary yield of PSII photochemistry (Fv/F_0) and the maximum/potential quantum efficiency of PSII (Fv/Fm) in fertilzed plants showing the high level of growth.





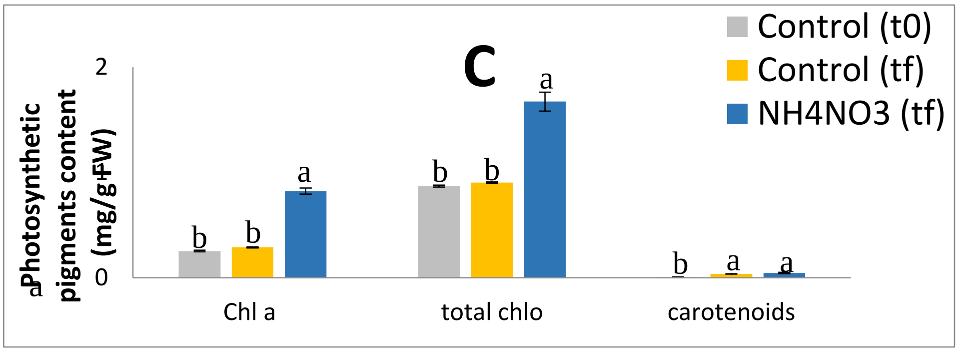


Figure 2: The chlorophyll fluorescence parameters Fo, Fm, (A), Fv/F0 and Fv/Fm (B) and photosynthtetic pigments contents (C) in *Moringa oleifera* grown on different nutritive media (control and NH_4NO_3 fertilisation). (t0: time zero; tf: final time).

Recently, Song et al, (2019) reported that nitrogen application improved chlorophyll fluorescence processes of oat plants. Likewise, Nasraoui and Gouia (2014), and Ullah et al, (2020) reported that nitrogen fertilezer application affects largely photosynthesis pocess. In fact, photosynthesis enhancement was directly linked to photosynthetic pigments tenors in leaves tissues. Figure 2C demonstareted thet referring to control, fertilization resulted in a greater than 80% increase in total chloropyll. The photosynthetic pigments amounts in leaves were closely related to nitrogen fertilizer (Zebarth et al., 2002), therefore, availability of chlorophylls enhance photosynthesis resulting in plant growth (Yuan, 2017; Rodrigo et al., 2019; Peng, Zhang et al., 2019; Chen et al., 2021; Gitelson et al., 2003).

Far from, and because there is no doubt that nitrogen disponibility to plants is one of the major factors affecting plant growth and thier richness in bioactives compounds crucial to cover vital humain needs (Yadav et al., 2017; Fathi and Zeidali, 2021; Amin, 2022). Furthermore, the observed rise in crude protein content could potentially be attributed to rise in crude protein content that seemed a result of availability of nitrogen stimulating its uptake and distribution in different plant organs (Ngwenya et al., 2022). Regarding organic compounds, Figure 3 displayed rise of proline and soluble sugars contents under ammonium nitrate fertilization condition. Similar results were published by Li et al, (2012), Zahra et al, (2021) and Amin (2022).

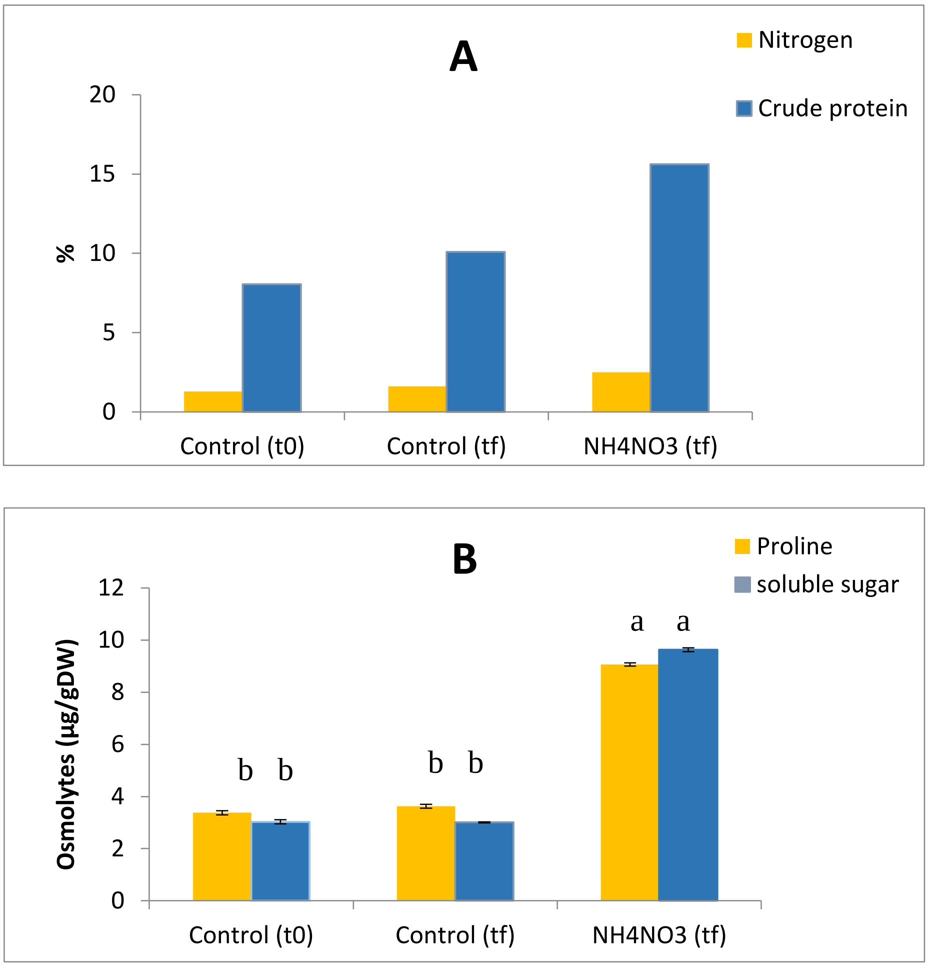


Figure 3: Variation of nitrogen and crude proteins (A), and osmolytes level (B) in *Moringa oleifera* grown on different nutritive media (control and NH_4NO_3 fertilisation). (t0: time zero; tf: final time).

Coming back to the reality that *Moringa oleifera* has immense value and is remarkably versatile in medicinal and pharmaceutical applications, we must remember that all these characteristics are in relationship with its richness in secondary metabolites (Mohammed et al., 2015). About it, our data (Figure 3) demonstrated how foliar tenor in total phenolic compounds, flavonoids, and condensed tannins were significantly elevated by ammonium nitrate fertilizer application. In fact, in control plants, the level of total polyphenols, flavonoids and tannins increased with plant age from November 2022 (t0) to December 2023 with about 10%, 16% and 13%. With plant fertilization, the levels of total polyphenols, flavonoids, and tannins increased by over two folds in comparison to the control group (Figure 4).

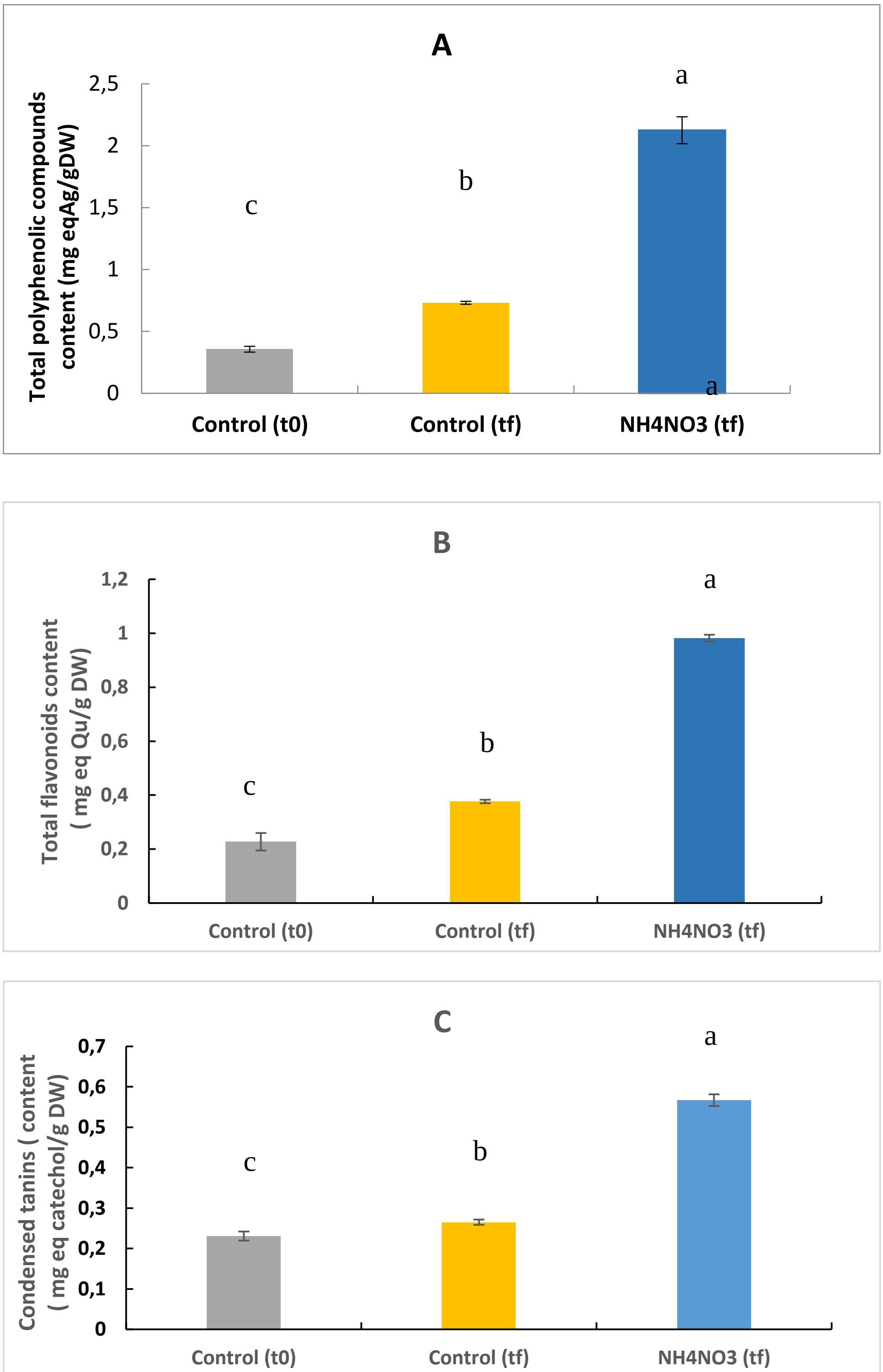


Figure 4: Variation of total polyphenolic compounds (A) total flavonoids (B) and condensed tanins (C) in *Moringa oleifera* grown on different nutritive media (control and NH_4NO_3 fertilisation). (t0: time zero; tf: final time).

However, positive correlations were observed between proteins and secondary metabolites (including flavonoids and tannins) contents in Moringa leaves (Table 4). In accordance with Litvaak et al, (1996) and Mutua et al, (2021), increased nutrient concentration in leaves may lead to enhanced carbon fixation and protein synthesis, resulting in raised production of secondary metabolites. In contrast to this context, Bustamante et al, (2020), reported a negative correlation between N fertilization and secondary metabolites in *Rosmarinus officinalis*.

Positive correlation (r= 0.944) observed between Fv/Fm and ssoluble sugars (Table 4) which are products of photosynthesis and reflecting the rate of carbohydrate metabolism. In fact, availability of high nitrogen level which influences soluble sugar production is significantly pronounced in fertilized Moringa in which carbon assimilation was more pronounced than in control ones (Jianbo et al., 2020).

Highlights in our study demonstrated that nitrogen fertilizer plays a crucial role in *Moringa oleifera* growth. Improving fertility of soil by its amendment by nitrogen fertilizer (NH_4NO_3) corresponds to an effective key strategy for improving biochemical quality of *Moringa oleifera* biomass.

Table 4: Pearson's correlation coefficients of morphological parameters changes and biochemical measures in nitrogen- fertilized *M. oleifera leaves*

Correlations

		Treatments	Total leaves frech weight	Total leaves drywieght	Chlo a content	Total Chlo content	Carotenoides content	Fv/Fo	Fv/Fm	Proline content	Soluble sugars content	Nitrogen content	Crude proteines	Total phenolic compounds content	Total flavonoids content	Condensed tanins content
Treatments	Pearson Correlation	1	,986**	,952**	,890**	,877**	,945**	,954**	,951**	,883**	,865**	,963**	,963**	,882**	,896**	,907**
	Sig. (2-tailed)		,000	,000	,000	,000	,000	,000	,000	,000	,000	,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Total leaves frech weight	Pearson Correlation	,986**	1	,985**	,952**	,936**	,878**	,977**	,953**	,947**	,934**	,993**	,993**	,942**	,955**	,962**
	Sig. (2-tailed)	,000		,000	,000	,000	,000	,000	,000	,000	,000	,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Total leaves drywieght	Pearson Correlation	,952**	,985**	1	,981**	,981**	,817**	,966**	,929**	,981**	,973**	,994**	,994**	,984**	,986**	,988**
	Sig. (2-tailed)	,000	,000		,000	,000	,001	,000	,000	,000	,000	,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Chlo a content	Pearson Correlation	,890**	,952**	,981**	1	,985**	,701*	,947**	,887**	,998**	,997**	,975**	,975**	,992**	,997**	,997**
	Sig. (2-tailed)	,000	,000	,000		,000	,011	,000	,000	,000	,000	,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Total Chlo content	Pearson Correlation	,877**	,936**	,981**	,985**	1	,706*	,921**	,865**	,991**	,990**	,963**	,963**	,998**	,991**	,987**
	Sig. (2-tailed)	,000	,000	,000	,000		,010	,000	,000	,000	,000	,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Carotenoides content	Pearson Correlation	,945**	,878**	,817**	,701*	,706*	1	,831**	,868**	,694*	,667*	,830**	,830**	,706*	,717**	,732**
	Sig. (2-tailed)	,000	,000	,001	,011	,010		,001	,000	,012	,018	,001	,001	,010	,009	,007
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Fv/Fo	Pearson Correlation	,954**	,977**	,966**	,947**	,921**	,831**	1	,987**	,936**	,926**	,976**	,976**	,931**	,955**	,955**
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,001		,000	,000	,000	,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Fv/Fm	Pearson Correlation	,951**	,953**	,929**	,887**	,865**	,868**	,987**	1	,875**	,861**	,944**	,944**	,875**	,902**	,903**
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,000	,000		,000	,000	,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Proline content	Pearson Correlation	,883**	,947**	,981**	,998**	,991**	,694*	,936**	,875**	1	,999**	,971**	,971**	,996**	,997**	,996**
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,012	,000	,000		,000	,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Soluble sugars content	Pearson Correlation	,865**	,934**	,973**	,997**	,990**	,667*	,926**	,861**	,999**	1	,962**	,962**	,995**	,995**	,994**
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,018	,000	,000	,000		,000	,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Nitrogen content	Pearson Correlation	,963**	,993**	,994**	,975**	,963**	,830**	,976**	,944**	,971**	,962**	1	1,000**	,968**	,976**	,983**
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,001	,000	,000	,000	,000		0,000	,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Crude proteines	Pearson Correlation	,963**	,993**	,994**	,975**	,963**	,830**	,976**	,944**	,971**	,962**	1,000**	1	,968**	,976**	,983**
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,001	,000	,000	,000	,000	0,000		,000	,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Total phenolic compounds content	Pearson Correlation	,882**	,942**	,984**	,992**	,998**	,706*	,931**	,875**	,996**	,995**	,968**	,968**	1	,996**	,994**
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,010	,000	,000	,000	,000	,000	,000		,000	,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Total flavonoids content	Pearson Correlation	,896**	,955**	,986**	,997**	,991**	,717**	,955**	,902**	,997**	,995**	,976**	,976**	,996**	1	,997**
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,009	,000	,000	,000	,000	,000	,000	,000		,000
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
Condensed tanins content	Pearson Correlation	,907**	,962**	,988**	,997**	,987**	,732**	,955**	,903**	,996**	,994**	,983**	,983**	,994**	,997**	1
	Sig. (2-tailed)	,000	,000	,000	,000	,000	,007	,000	,000	,000	,000	,000	,000	,000	,000	
	N	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

CONCLUSIONS

In conclusion, ammonium nitrate fertilizer contributes significantly in the growth improvement of Moringa oleifera and its biochemical quality amelioration. Inorganic fertilizer (NH_4NO_3) application regulates chlorophyll fluorescence intensity, thereby strengthens and promotes photosynthesis process. There for, it stimulates carbohydrate metabolism, which is fundamental process ensuring energy supply to living cells. The mineral nitrogen fertilizer addition promotes foliar content in crucial bioactive molecules keys of *Moringa oleifera* leaves values.

REFERENCES

Amin, F. 2022. Role of nitrogen (N) in plant growth, photosynthesis pigments, and N use efficiency: A review Agrisost. 28: 1-8. Africa: a review. Experimental Agriculture. 51:501-521.

Arnon, D.I., Allen, M.B., Whatleyer, F.R. 1956. Photosynthesis by isolated chloroplasts. IV. General concept and comparison of three photochemical reactions, Biochemistry and Biophysics Acta. 20: 449-61.

Azeem, M., Pirjan, K., Qasim, M., Mahmood, A., Javed, T., Muhammad, H., Rahimi, M. 2023. Salinity stress improves antioxidant potential by modulating physio-biochemical responses in Moringa oleifera Lam. Scientific Reports. 13: 2895.

Ba, K., Tine, E., Destain, J., Cisse, N.,Thonart, P. 2010. Étude comparative des composés phénoliques, du pouvoir antioxydant de différentes variétés de sorgho sénégalais et des enzymes amylolytiques de leur malt, Biotechnolo. Agro. Soc. Environ. 14:131-139.

Bates, L., Waldren, R., Teare, I. 1973. Rapid determination of free proline for waters tress studies, Plant Soil. 39:205–7.

Nogues, I. 2020. Effects of soil fertilization on terpenoids and other carbon-based secondary metabolites in Rosmarinus officinalis plants: a comparative study. Plants. 9: 830.

Chen, M., Zhu, K., Tan, P., Liu, J., Xie, J., Yao, X., Peng, F. 2021. Ammonia–nitrate mixture dominated by NH4+–N promoted growth, photosynthesis and nutrient accumulation in Pecan (Carya illinoinensis). Forests. 12: 1808.

Dehpour, A.,A., Ibrahimzadeh, M.,A., Fazel, N.,S., Mohammad, N.,S. 2009. Antioxydant activity of the methanol extract of Ferula assafoetida and its essential oil composition, Grasas Y Aceites. 60:405-412.

Dubois, M., Gilles, K.,A., Hamilton, J.,K., Reders, P., A., Smith, F. 1956. Colorimetric method for determination of sugars and related substances. Annals of Biochemistry. 28:350-356.

Fathi, A., Zeidali, E. 2021. Conservation tillage and nitrogen fertilizer: a review of corn growth, yield and weed management. *Cent Asian Journal of Plant Science Innovation.*3: 121–142.

Gad, N., Sekara, A., Abdelhamid, M.T. 2019. The potential role of cobalt and/or organic fertilizers in improving the growth, yield, and nutritional composition of Moringa oleifera. Agronomy. 9: 862.

Gitelson, A.A., Gritz, Y., Merzlyak, M.N. 2003. Relationships between leaf chlorophyll content and spectral reflectance and algorithms for non-destructive chlorophyll assessment in higher plant leaves. Journal of Plant Physiology. 160:271–282.

Hafeez, N. , Pengcheng, D., Aixia, R., Min, S. Zhiqiang, G. 2023. Effects of nitrogen fertilizer on photosynthetic characteristics and yield. Agronomy. 13: 1550.

Jianbo, S., Li, W., Li, C., Chang, W., Zhang, S., Zeng, Y., Zeng, C., Peng, M. 2020. Effect of different rates of nitrogen fertilization on crop yield, soil properties and leaf physiological attributes in Banana under subtropical regions of China. Front Plant Science. 11:613760.

Karín, E., Coello, F.J., Martínez-Villaluenga, C., Cartea, M.A., Rosaura, A., Elena, P. 2020. Potential of germination in selected conditions to improve the nutritional and bioactive properties of Moringa (*Moringa oleifera L.*). Foods.1-15.

Kayuki, C., Mohammed, B.M., Maman, N. 2017. Niger Fertilizer use optimization in Sub-Saharan Africa. *Eds* Charles S. Wortmann and Keith Sones.9-19.

Khalaj, M.A., Noroozisharaf, A. 2020. Efficiency of ammonium and nitrate ratios on macronutrient content and morphological properties of Gerbera jamesonii cut flower. Agriculturae Conspectus Scientificus. 85: 281-289.

Korodjouma, O., Idriss, S., Alimata, A., Bandaogo, S.O., Adama, S., Zakaria, G., Sansan, Y., Pascal, Y., Tahibou, P. 2017. Optimizing fertilizer use within an integrated soil fertility management framework in burkina faso fertilizer use optimization in sub-saharan Africa Charles S. Eds Wortmann and Keith Sones. 40-51.

Li, J.W., Ding, S.D. Ding, X.L. 20. Comparison of antioxidant capacity of extracts from five cultivars of Chinese jujub. Proc of Biochemistry. 40:3607– 3613.

Li, Y., Yong-an L.I.U., Cai-yong, X.I.E., Xue, J.I.AN.G., Yong-jie, W.N.G., Yin-hua, L.I. Ting-xing, H.U. 2012. Effects of drought stress and nitrogen fertilization rate on the accumulation of osmolytes in Jatropha curcas seedlings. Yingyong Shengtai Xuebao. 233.

Litvak, M.E., Loreto, F., Harley, P.C., Sharkey, T.D., Monson, R.K. 1996. The response of isoprene emission rate and photosynthetic rate to photon flux and nitrogen supply in aspen and white oak trees. Plant Cell and Environement. 19:549–559.

Liu, C.A., Li, F.R., Zhou, L.M., Zhang, R.H., Lin, S.L., Wang, L.J. 2013. Effect of organic manure and fertilizer on soil water and crop yields in newly-built terraces with loess soils in a semi-arid environment. Agriculture Water Management. 117: 123–132.

Mason, S.C., Maman, N., Palé, S. 2015. Pearl millet production practices in semi-arid west

Mohammed, S., Manan, F.A. 2015. Analysis of total phenolics, tannins and flavonoids from Moringa oleifera seed extract. Journal of Chemical and Pharmaceutical Research. 7:132-135.

Mutua, C.M., Ogweno, J.O., Gesimba, R.M. 2021. Effect of NPK fertilizer rates on secondary metabolites of pepino melon (*Solanum muricatum* Aiton). Journal of Horticulture and Forestry. 13: 25-34.

Nasraoui, H.A., Gouia, H. 2014. Photosynthesis sensitivity to NH_4^+-N change with nitrogen fertilizer type**.** Plant Soil Environmental. 6: 274–279.

Ndagi, H.I., Agera, S.I.N., Chenge, I.B., Tunku, P.I. 2023. Effect of organic and inorganic fertilizer treatments on *Moringa Oleifera* seedlings Lafia, Nasarawa State, Nigeria. Journal of Research in Forestry, Wildlife and Environment. 15: 19-26.

Padayachee, B., Baijnath, H. J. S. 2020. An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of Moringa oleifera. South African Journal of Botany. 129:304-316.

Patson, N., Catherine, K., James, M., Francis, T., Bitrus, T., Mohammed, K., Korodjouma, O., Rusanganwa, A. Cyamweshi, M. K. Nouri, Wondimu, B., Charles ,S. 2017. Fertilizer Use Optimization: Principles and Approach. Integrated soil fertility management in sub-saharan africa fertilizer use optimization in sub-saharan Africa Charles S. Eds Wortmann and Keith Sones.25-39.

Pearson, D. 1976. Chemical Analysis of Foods. 7th Edition, Churchhill Livingstone, London.

Pedersen, I.F., Sørensen, P., Rasmussen, J., Withers, P.J., Rubæk, G.H. 2019. Fertilizer ammonium: nitrate ratios determine phosphorus uptake by young maize plants. Journal of Plant Nutrition and Soil Science. 182: 541-551.

Phinehas, T.J., Khare, N., Paruchuri, R.G., Vijaykumar, R. 2022. Effect of Organic Manures and Inorganic Fertilizers on Growth and Yield of Chickpea (Cicer Arietinum L.) under Moringa Agroforestry System.

Rodrigo, O. M.T., Porfirio, J., Ronald, E. O. C., Manuel, S., Iran, A. T., Gelacio, A.S. 2019. Nitrogen and chlorophyll status of Romaine Lettuce using SPAD and at LEAF readings. Not Bot Horti Agrobo. 47:15835-11589.

Sadak, M.S., Abdalla, A.M., AbdElhamid, E.M., Ezzo, M.I. 2020. Role of melatonin in improving growth, yield quantity and quality of Moringa oleifera L. plant under drought stress. Bulletin of the National Research Centre. 44: 1-13.

Simangele, C. Ngwenya, O. T., Edje, Bernard N., Dlamini, Tamado, T. 2022.

Song, X., Zhou, G., Ma, B.L., Wu, W., Ahmad, I., Zhu, G., Jiao, X. 2019. Nitrogen application improved photosynthetic productivity, chlorophyll fluorescence, yield and yield components of two oat genotypes under saline conditions. Agronomy. 9: 115.

Souza, E.F., Soratto, R.P., Sandaña, P., Venterea, R.T., Rosen, C.J. 2020. Split application of stabilized ammonium nitrate improved potato yield and nitrogen-use efficiency with reduced application rate in tropical sandy soils. Field Crops Research. 254:107-847.

Sreelatha, S., Padma, P.R. 2009. Antioxidant activity and total phenolic content of Moringa oleifera leaves in two stages of maturity. Plant foods for human nutrition. 64: 303-311.

Ullah, I., Hanping, M., Javed, Q., Rasool, G., Ali, M., Azeem, A., Saif, M. 2020. Nitrogen Fertilization Effects on Growth, Leaf Gas Exchange and Chlorophyll Fluorescence of Brassica juncea. International Journal of Agriculture and Biology. 24: 1070-1076.

Vongsak, B., Sithisarn, P., Gritsanapan, W. 2014. Simultanioues HPLC quantitave analysis of active compounds in leaves of Moringa oleifera Lam Journal of chromatography science. 52: 641-645.

Yadav, M.R., Kumar, R., Parihar, C.M., Yadav, R.K., Jat, S.L., Ram, H., Meena, R.K., Singh, M., Verma, A.P., Kumar, U., Ghosh, A., Jat, M.L. 2017. Strategies for improving nitrogen use efficiency: A review. Agriculture Revue. 38: 1-8.

Yan, L., Xu X., Xia, J. 2019. Different impacts of external ammonium and nitrate addition on plant growth in terrestrial ecosystems: A meta-analysis. Science of the total environment. 686: 1010-1018.

Yuan, L. , Yujie, Y. , Shan, L., Jie, W., Shidong, Z., Guohu, C., Jinfeng, H., Chenggang, W. 2017. Influence of high temperature on photosynthesis, antioxidative capacity of chloroplast, and carbon assimilation among heat-tolerant and heat-susceptible genotypes of non heading Chinese Cabbage. Horticulture Science. 52:1464–1470.

Zahra, R., Mohammadian, R.N., Sadeghi, S.M., Ebrahim, A., Doroudian, H.R. 2021. Effect of drought stress and different levels of nitrogen and potassium fertilizers on the accumulation of osmolytes and chlorophyll in rice (Oryza sativa L.). Gesunde Pflanzen. 73: 287-296.

Zanella, L., Gismondi, A., Di Marco, G., Braglia, R., Scuderi, F., Redi, E.L., Canini, A. 2019. Introduction of antioxydants metabolites in *Moringa oleifera* callus by abiotic stresses. Journal of natural products. 82: 2379-2386.

Zebarth, B.J., Younie, M., Paul, J.W., Bittman, S. 2002. Evaluation of leaf chlorophyll index for making fertilizer nitrogen recommendations for silage corn in a high fertility environment. Communications in soil science and slant analysis. 33:665-684.

Zhang, J., Lv, J., Dawuda, M.M., Xie, J., Yu, J., Li, J., Gan, Y. 2019. Appropriate ammonium-nitrate ratio improves nutrient accumulation and fruit quality in pepper (*Capsicum annuum* L.). Agronomy. 9: 683.

STRAIN EFFECT ON HOLE-CONFINED PHONON SCATTERING RATES IN $Al_{0.25}Ga_{0.75}As/GaAs/Al_{0.25}Ga_{0.75}As$ QUANTUM WELLS

Mohamed Boumaza
Teacher Education college of Setif Messaoud Zeghar, Department of sciences, El Eulma, 19600, Algeria
Corresponding author e-mail: mboumazaphy1@gmail.com

ABSTRACT

This study presents a theoretical investigation of hole-confined polar optical phonon scattering rates in symmetric $Al_{0.25}Ga_{0.75}As/GaAs/Al_{0.25}Ga_{0.75}As$ quantum wells. We compute intrasubband heavy hole-confined phonon scattering rates in the quantum well using Fermi's Golden Rule in conjunction with the dielectric continuum model. Numerical results demonstrate that these scattering rates are reduced by strain via tensile hydrostatic strain and tensile biaxial strain. We compare scattering rates for quantum wells grown along the [001] and [011] directions. Our findings show increased scattering rates for the [011] growth direction compared to the [001] orientation.

Keywords: Quantum Well, Confined Phonon, Semiconductor, Strain

INTRODUCTION

Strain in semiconductor physics is an important tool to alter the band structure and, consequently, modify the electronic and optical properties of materials. Strain can improve optoelectronic devices such as quantum well lasers. Strain can be caused by phonons or induced by a mismatched lattice in heterostructures, as well as by external application (Sun, et al., 2007).

In this work, we study the interaction of holes with confined phonons in a symmetric quantum well, $Al_{0.25}Ga_{0.75}As/GaAs/Al_{0.25}Ga_{0.75}As$, as well as the effect of stress on this interaction, considering both hydrostatic tensile and biaxial tensile strain (Ehrhardt. Et al., 2014). Moreover, we calculate scattering rates for two quantum well growth directions, [001] and [011], respectively. Finally, we study the effect of valence band anisotropy on scattering rates, concluding with a summary.

MATERIAL AND METHOD

Using the empirical band structure method, we calculate the valence band structure, employing Bloch states as basis functions for the Schrödinger equation. The 6×6 Luttinger-Kohn Hamiltonian is implemented via the k·p method, incorporating heavy holes, light holes, and split-off subbands while accounting for spin-orbit interaction (Willatzen, et al. , 2009; Boumaza et al. 2014)

Fermi's golden rule is used to determine the scattering rate of a hole from a state with in-plane wave vector k_i in subband i to all possible hole states in subband f with wave vector k_f . The rule can be expressed as (Ridley 1997; Boumaza et al., 2022)

$$\gamma_{i\to f} = \frac{2\pi}{\hbar}\int |M(f,i)|^2\,\delta\left(E_f \pm \hbar\omega - E_i\right)dN_f \qquad (1)$$

In this case, the number of final hole states is denoted by N_f , the initial and final hole state energies are E_i and E_f, respectively. The matrix element that connects the hole states engaged in the scattering process is denoted by $M(i, f)$. Equation (1) gives us

$$\gamma_{i\to f} = \frac{1}{2\pi\hbar}\frac{k_f}{\frac{\partial E_f}{\partial k}}\left(N_q + \frac{1}{2} \pm \frac{1}{2}\right)\Gamma_{if} \qquad (2)$$

Where Nq is the phonon occupation number and the function Γ_{if} is the overlap integral of the hole wave function and the phonon potential, which is given by

$$\Gamma_{if} = \sum_m \int \left|\int \langle f, k_f | e\Phi_m(z) | i, k_i \rangle dz\right|^2 d\theta \qquad (3)$$

Here, e represents the electron charge. Equation (3) is calculated numerically, taking into account the conservation of energy and momentum. The notation for the phonon potential is as follows (Mori et al., 1989).

$$\Phi_m(z) = A_C cos(\frac{m\pi z}{L}) \qquad m=1,3,5,.. \qquad (4)$$

$$\Phi_m(z) = A_C \sin(\frac{m\pi z}{L}) \qquad m=2,4,6,.. \qquad (5)$$

Here, L is the quantum well width, and AC is the normalization constant.

Table 1. Parameters used in this work (Boyer-Richard et al., 2011; Vurgaftman et al., 2001; Adachi 1985)

Parameter	Unit	GaAs	AlAs
γ_1		6.85	3.69
γ_2		2.1	0.79
γ_3		2.9	1.4
Δ	eV	0.341	0.28
E_g	eV	1.424	2.671
a_v	eV	-1.16	-2.47
$\hbar\omega_{LO}$	eV	0.03625	0.05009

RESULTS AND DISCUSSION

Figure 1 shows the structure of the valence band of heavy holes as a function of the wave vector *k* in the (xy) plane for a quantum well with a width of 30 Å in a $Al_{0.25}Ga_{0.75}As/GaAs/Al_{0.25}Ga_{0.75}As$ system, where we clearly observe an anisotropy that becomes more pronounced as we move away from the center of the Brillouin zone.

Figure 2 shows the scattering rates for intrasubband heavy hole absorption of confined phonon, both without strain and with hydrostatic tensile and biaxial tensile strain. We observe that the scattering rates decrease rapidly with increasing hole energy, from a maximum value of $0.57x10^{12}$ s^{-1} to $0.43x10^{12}$ s^{-1}. After this point, the variation with hole energy becomes weaker. The effect of strain induces a reduction in scattering rates, particularly for low-energy heavy holes. However, for tensile biaxial strain, the reduction is more significant, reaching up to 25 %.

Despite the dependence of scattering rates on the variation of the density of states and the integral overlap, the behavior of the scattering rate is greatly influenced by the variation in the overlap integral, as shown in Figure 3. When warping in the subband structure is fully taken into account, Figure 4 displays the intrasubband scattering rate due to the confined phonon as a function of the initial 2D wave vector, where we can clearly observe a strong anisotropy at high energies of heavy holes. This anisotropy originates from the anisotropy in the band structure of heavy holes.

In Figure 5, we calculate the intrasubband scattering rates for confined holes in two growth directions, [001] and [011]. Our results show an increase in scattering rates for the [011] direction, which is attributed to the higher density of states and the increased overlap integral.

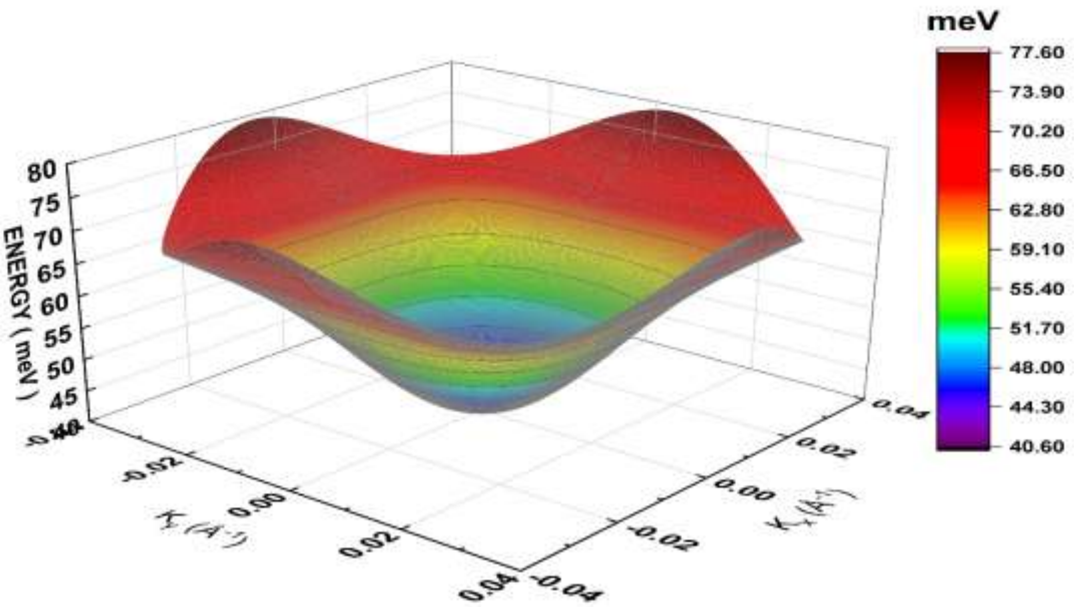


Figure 1 Valence band structure for the heavy hole subband in a quantum well with a width of 30 Å

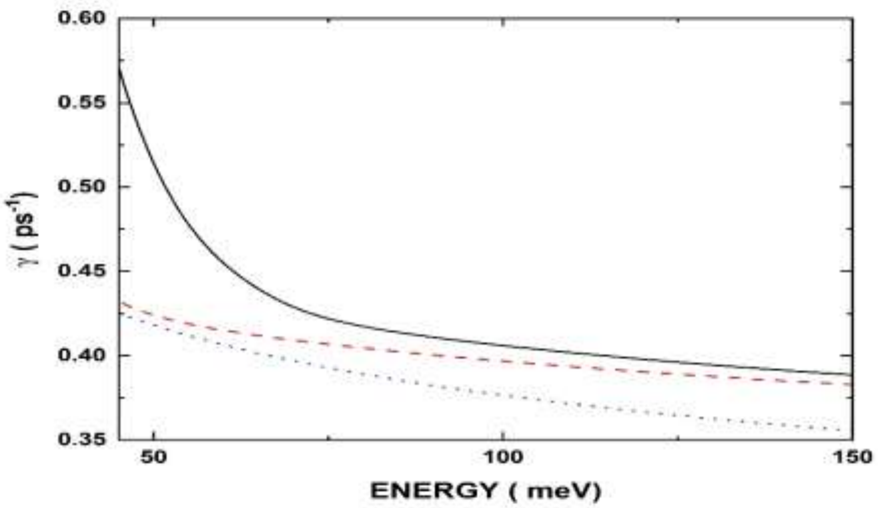


Figure 2 Intrasubband scattering rate with confined phonons as a function of hole energies, where the solid lines represent the case without strain, and the dashed and dotted lines correspond to tensile hydrostatic strain and tensile biaxial strain, respectively.

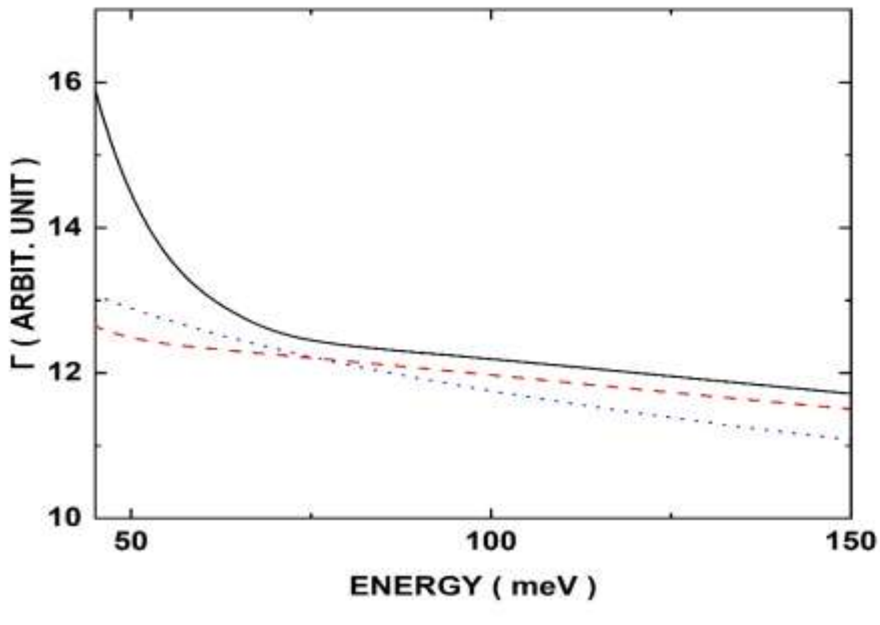


Figure 3. The overlap integral Γ_{if} as a function of hole energies, where the solid lines represent the case without strain, and the dashed and dotted lines correspond to tensile hydrostatic strain and tensile biaxial strain, respectively.

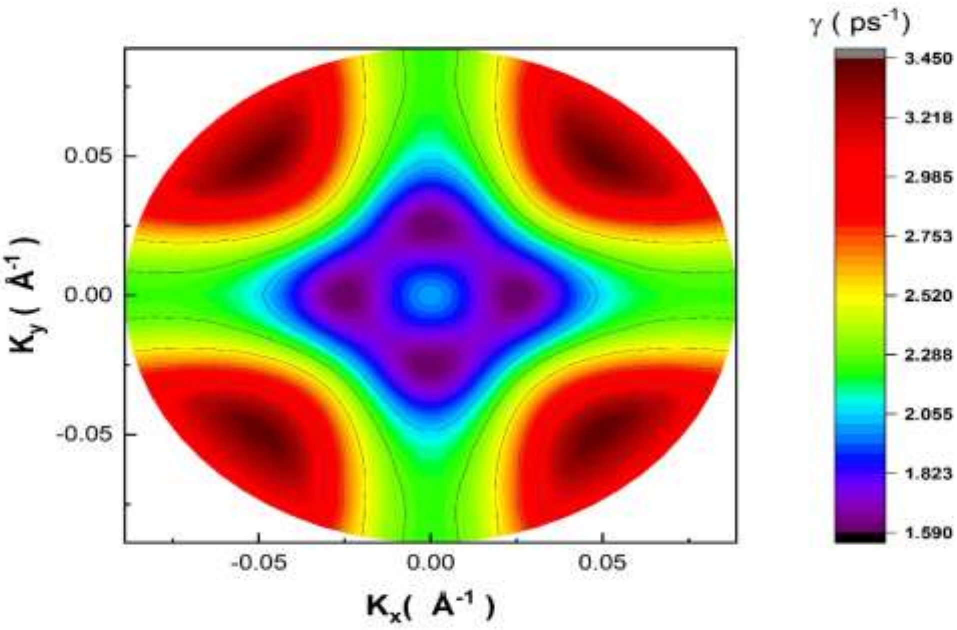


Figure 4. Intrasubband scattering rates with confined phonon absorption as a function of the hole wave vector k in polar coordinates and for L = 30 Å.

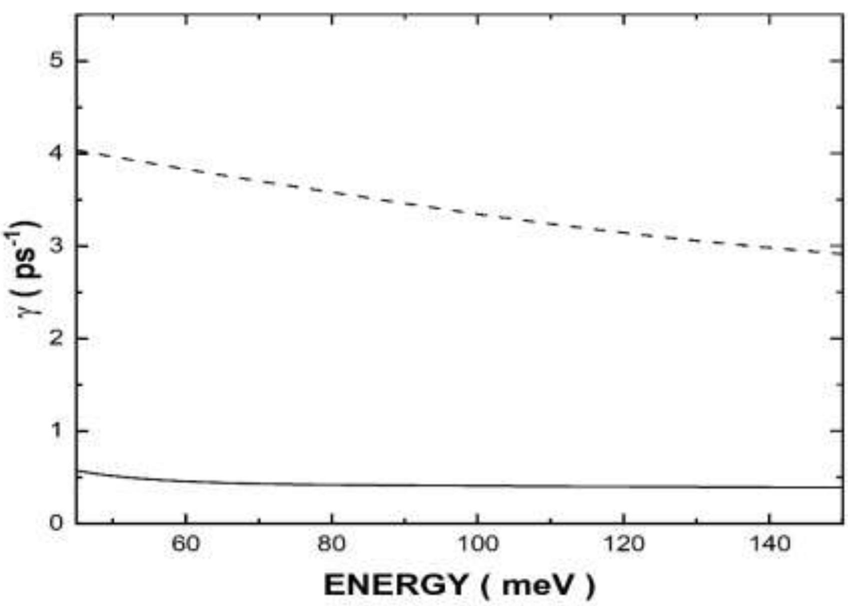


Figure 5. Intrasubband scattering rates as a function of hole energies, where the solid lines represent the case when the growth direction is [001], and the dashed lines correspond to the [011] growth direction.

CONCLUSIONS

This study provides a comprehensive analysis of hole-confined phonon scattering rates in $Al_{0.25}Ga_{0.75}As/GaAs/Al_{0.25}Ga_{0.75}As$ quantum wells, considering the effects of strain on these interactions. Our results demonstrate that tensile hydrostatic and biaxial strains significantly reduce scattering rates, especially at lower hole energies, contributing to enhanced hole mobilities. We also observe that the scattering rates increase along the [011] growth direction compared to the [001] orientation, primarily due to the increased density of states and the overlap integral. Additionally, the strong anisotropy in the valence band structure and its impact on scattering rates were highlighted. These findings contribute to a deeper understanding of strain effects on hole-phonon interactions in quantum wells, offering valuable insights for the design and optimization of advanced semiconductor devices.

REFERENCES

Sun, Y., Thompson, S. E., & Nishida, T. (2007). Physics of strain effects in semiconductors and metal-oxide-semiconductor field-effect transistors. Journal of Applied Physics, 101(10).

Ehrhardt, M., & Koprucki, T. (Eds.). (2014). Multi-Band Effective Mass Approximations: Advanced Mathematical Models and Numerical Techniques (Vol. 94). Springer.

Willatzen, M., & Voon, L. C. L. Y. (2009). The kp method: electronic properties of semiconductors (Vol. 1). Springer-Verlag Berlin Heidelberg.

Boumaza, M., & Lamari, S. (2014). Anisotropic intrasubband hole scattering by polar optical phonon modes in thin GaAs/AlxGa1−xAs quantum wells. Superlattices and Microstructures, 72, 156-163.

Ridley, B. K. (1997). Electrons and phonons in semiconductor multilayers (No. 5). Cambridge University Press.

M. Boumaza et Y. Boumaza. Anisotropic intrasubband hole Scattering rates by confined polar optical phonon in AlxGa1-xAs/GaAs/AlyGa1-yAs asymmetric quantum wells (2022). Advances in Science, Technology and Engineering Systems Journal Vol. 7, No. 3, 82-86

Mori, N., & Ando, T. (1989). Electron–optical-phonon interaction in single and double heterostructures. Physical Review B, 40(9), 6175.

Boyer-Richard, S., Raouafi, F., Bondi, A., Pedesseau, L., Katan, C., Jancu, J. M., & Even, J. (2011). 30-band k· p method for quantum semiconductor heterostructures. Applied Physics Letters, 98(25).

Vurgaftman, I., Meyer, J. A. R., & Ram-Mohan, L. R. (2001). Band parameters for III–V compound semiconductors and their alloys. Journal of applied physics, 89(11), 5815-5875.

Adachi, S. (1985). GaAs, AlAs, and Al x Ga1− x As: Material parameters for use in research and device applications. Journal of applied physics, 58(3), R1-R29.

DETERMINATION OF ANTIBIOTIC RESISTANCE PROFILES OF *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM DIABETIC FOOT INFECTIONS

Zeynep ERDEM AYNUR[1], Bülent BOZDOĞAN[1,2], Bülent ERTUĞRUL[3],Gamze BAŞBÜLBÜL[1,4]

[1]Aydın Adnan Menderes University ADUREDPROM Laboratory
[2] Aydın Adnan Menderes University Faculty of Medicine, Department of Medical Microbiology [3] Aydın Adnan Menderes University Faculty of Medicine, Department of Infectious Diseases
[4] Aydın Adnan Menderes University, Faculty of Science, Department of Biology, Department of Molecular Biology

ABSTRACT

One of the important complications of diabetes is diabetic foot infections accompanied by neuropathy and vascular problems. These infections, especially caused by resistant bacteria, are among the most important etiological causes of foot amputations. *Pseudomonas aeruginosa* is the most common cause of diabetic foot infections in our country. The widespread and multidrug resistance seen in this bacterium poses a global threat. In this study, the antibiotic resistance profile of 100 *Pseudomonas aeruginosa* strains isolated from diabetic foot infections was determined by E-test method. **Materials and Methods:** In our study, a total of 100 *Pseudomonas aeruginosa* strains isolated from diabetic foot infections were used. The susceptibilities of different antibiotics (piperacillin, piperacillin+tazobactam, ceftazidime, meropenem, ticarcillin+clavulanate, cefepime, cefpirome, aztreonam, imipinem, amikacin, ciprofloxacin and tigecycline) in *Pseudomonas aeruginosa* isolates were determined using the E-test method**. Results:** While two of the *Pseudomonas aeruginosa* strains isolated from diabetic foot infections were resistant to all the tested antibiotics, all the isolates except one of the isolate were found to be intermediate or resistant to at least one antibiotic. The most common resistance rate among *Pseudomonas aeruginosa* strains evaluated within the scope of our study was Ticarcillin-Clavulanate (60%), followed by Ciprofloxacin (48%), Aztreonam (45%), Piperacillin (30%), Piperacillin-Tazobactam (28%), Ceftazidime. (22%), Imipenem and Meropenem (15%), Amikacin (13%). The least resistance was found against Cefepime (4%). Since there is no breakpoint value in the CLSI data of tigecycline and cefpirome, the resistance rate could not be determined. **Discussion and Conclusion:** *Pseudomonas aeruginosa*, which has multidrug resistance, is being isolated at increasing rates from diabetic foot infections in our country. According to previous studies, although the resistance situation was generally similar, it was observed that sensitivity to carbapenems was higher and the resistance to cefepime was very low. Monitoring sensitivity to antibiotics according to isolation locations is important in directing empirical treatments.

INTRODUCTION

Diabetes is a significant public health issue, and the number of people affected by the disease is increasing daily (Jneid et al., 2017). Diabetes can lead to various complications such as kidney failure, heart attack, blindness, stroke, and lower extremity amputations. Diabetic foot ulcers are a major global health concern and are one of the most common conditions among diabetic patients. Diabetic foot ulcer is a primary reason for the hospitalization of diabetic patients. Approximately 25% of infected wounds do not heal, which can result in the amputation of the lower leg (Iraj et al., 2013).

Recent studies indicate that the causative agents of diabetic foot infections (DFIs) vary by country and geographic location (Hatipoğlu et al., 2014). In Europe, Gram-positive bacteria such as *Staphylococcus aureus* are predominantly isolated from diabetic foot ulcers. In temperate climate regions, including our country, as well as Asia and Africa, *Pseudomonas aeruginosa* is the most frequently encountered pathogen in these infections (Ertuğrul et al., 2017). For example, in a large case series study from India covering data from 1991 to 2008, Gram-negative agents accounted for 57.1% (with *P. aeruginosa* at 16.9%, being the most frequently detected agent), while Gram-positive agents were found at 40.6% (Ramakant et al., 2011). Similarly, a study from Kuwait reported a Gram-negative/positive ratio of 51%/32%, with *P. aeruginosa* at a high rate of 17.4% (Al Benwan et al., 2012). Studies conducted in our country also show that *P. aeruginosa* constitutes a high proportion of DFI agents, ranging from 15% to 20% (Ertuğrul et al., 2008; Ertuğrul et al., 2012; Ertuğrul et al., 2017). The most significant challenge in treating infections caused by this agent is antibiotic resistance. Another study from our country indicates that the treatment failure rate for DFIs caused by resistant agents is five times higher, amputation rates are increased, hospital stays are prolonged, and treatment costs are approximately twice as high compared to infections caused by susceptible agents (Ertuğrul et al., 2012).

As with most microorganisms, antibiotic resistance in *Pseudomonas aeruginosa* is increasing day by day. It is thought that inadequate infection control measures and inappropriate antibiotic use contribute to the rising antibiotic resistance rates in *Pseudomonas spp*.; this acquired antimicrobial resistance limits treatment options and complicates the management of infections. In the antimicrobial treatment of pseudomonal infections; aminoglycosides, antipseudomal cephalosporins, carbapenems, fluoroquinolones, beta-lactam/beta-lactamase inhibitors, monobactams, phosphonic acids, polymyxins are used (Magiorakos et al.,2012). Resistance of *P.aeruginosa* to antimicrobials may vary depending on time period, country, different geographical regions, hospitals and clinics of the same country (Avcıoğlu et al.2019, Çakmaklıoğulları ve Kuru, 2019, Shortridge et. al.,2019, Erdoğan et. al.,2021).

In this study, the antibiotic resistance profile of 100 Pseudomonas aeruginosa strains isolated from diabetic wound sites was examined according to CLSI criteria using the E-test method.

MATERIALS AND METHODS

Isolates Used:

A total of 100 *P. aeruginosa* isolates of diabetic foot ulcers origin from the collection of the Nazlı-Selim Eren Chronic Wound and Chronic Wound Infection Treatment Unit at Aydın Adnan Menderes University Hospital were used in the study.

E-Test Procedure:

P. aeruginosa isolates were cultured in Tryptic Soy Broth (TSB) and incubated at 37ºC for 18-24 hours. The density of the bacteria to be tested was adjusted to 0.5 McFarland. For this purpose, 100 µl from an overnight culture was suspended in 10 ml of 0.9% sterile saline solution (FTS), and the bacteria adjusted to a 0.5 McFarland density were spread on the surface of

Mueller-Hinton agar using a sterile swab. The plates were left to dry in an incubator at 37ºC for 10-15 minutes. Following this, E-test strips containing a specific antibiotic gradient (piperacillin, piperacillin + tazobactam, ceftazidime, meropenem, ticarcillin + clavulanic acid, cefepime, aztreonam, cefpirome, imipenem, amikacin, ciprofloxacin, and tigecycline) were placed on the agar surface with sterile forceps, with two different strips per plate. The plates were incubated at 37ºC for 18-24 hours. The next day, the Minimum Inhibitory Concentration (MIC) values were determined. The MIC value was considered the point where the inhibition ellipse intersected with the scale on the strip (Joyce et al., 1992).

RESULTS

E-test strips were used in determining the antibiotic sensitivities of *Pseudomonas aeruginosa* isolates in accordance with the manufacturer's instructions. MIC results of all tested antibiotics were obtained as examples shown in the picture (Figure 1). Critical values of antibiotics whose MIC values were determined by E-test according to CLSI 2023 are given in Table 1, and the E-test results performed with 100 *P. aeruginosa* are given in Table 2.

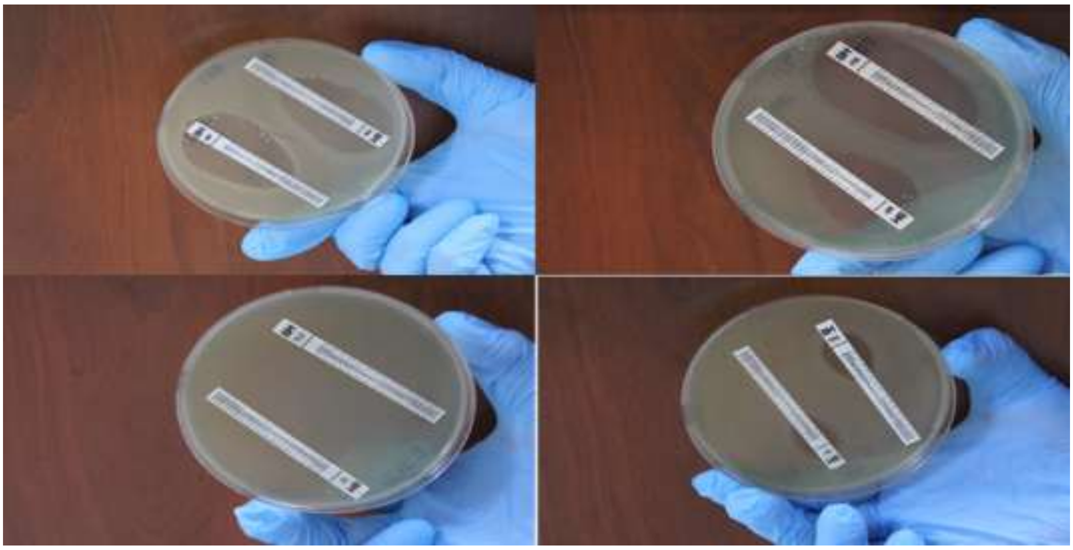


Figure 1. MIC values determined by E-test strips of different antibiotics

Table 1. Antibiotics used and their critical values (S: Sensitive, I: Intermediated R: Resistance)

Abbreviation	Antibiotic	Critical values
(TZ)	Ceftazidime	**S**≤8 **I**:16 **R**≥32
(AT)	Aztreonam	**S**≤8 **I**:16 **R**≥32
(MP)	Meropenem	**S**≤ 2 **I**:4 **R**≥8
(TGC)	Tigecycline	*There are no breakpoint values for P. aeruginosa.*
(TLC)	Ticarcillin + clavulanic acid	**S**≤16 **I**:32-64 **R**>64
(TPZ)	Piperacillin + tazobactam	**S**≤16 **I**:32-64 **R**>64
(AK)	Amikacin	**S**≤16 **I**:32 **R**≥64
(IMI)	Imipenem	**S**≤2 **I**:4 **R**≥8
(PP)	Piperacillin	**S**≤16 **I**:32-64 **R**>64
(PM)	Cefepime	**S**≤8 **I**:16 **R**≥32
(CL)	Ciprofloxacin	**S**≤0,5 **I**:1 **R**≥2
(CR)	Cefpirome	*There are no breakpoint values for P. aeruginosa..*

Table 2. E-test MIC results of *P. aeruginosa* isolates

Isolate	TZ	TLC	PP	TPZ	AT	CR	PM	MP	AK	CL	IMI	TGC
696	1.0	64.0	4.0	8.0	2.0	8.0	0.25	0.064	4.0	0.25	2.0	32.0
727	16.0	>256	8.0	16.0	>256	8.0	0.25	0.125	4.0	0.25	1.0	128.0
1039	2.0	>256	64.0	64.0	32.0	32.0	0.50	0.125	4.0	>256	0.50	128.0
2279	8.0	>256	>256	>256	128.0	>256	8.0	>256	128.0	>256	>256	128.0
337	0.5	32.0	4.0	8.0	2.0	8.0	0.25	0.32	2.0	0.25	1.0	16.0
1082	2.0	>256	8.0	16.0	>256	8.0	0.25	0.064	4.0	0.125	2.0	32.0
1668	2.0	128.0	8.0	16.0	>256	>256	1.0	0.125	4.0	>256	2.0	64.0
826	2.0	>256	32.0	16.0	4.0	8.0	0.25	0.064	4.0	4.0	0.50	16.0
526	2.0	>256	128.0	64.0	>256	>256	1.0	0.125	16.0	0.125	2.0	32.0
611	0.5	64.0	8.0	16.0	2.0	128.0	0.50	0.064	16.0	16.0	0.50	128.0
2277	2.0	>256	32.0	32.0	64.0	>256	1.0	0.064	32.0	>256	0.50	128.0
862	0.25	8.0	8.0	4.0	1.0	2.0	0.125	0.25	4.0	1.0	2.0	16.0
1930	0.5	>256	4.0	8.0	2.0	8.0	0.25	0.125	4.0	0.25	2.0	32.0
2256	2.0	>256	64.0	64.0	64.0	>256	1.0	>256	32.0	>256	1.0	>256
4468	>256	>256	>256	>256	>256	>256	8.0	16.0	64.0	>256	>256	32.0
2244	1.0	32.0	16.0	4.0	4.0	8.0	0.25	0.064	16.0	0.25	1.0	32.0
825	1.0	64.0	16.0	32.0	4.0	32.0	0.50	0.064	8.0	0.25	2.0	32.0
3940	0.50	32.0	4.0	4.0	2.0	8.0	0.125	0.032	4.0	0.25	0.25	32.0
2801	1.0	64.0	8.0	4.0	4.0	>256	>256	0.064	16.0	1.0	2.0	32.0
1666	1.0	64.0	4.0	8.0	4.0	>256	1.0	0.064	32.0	>256	2.0	128.0
3380	1.0	32.0	8.0	2.0	2.0	8.0	0.25	0.125	4.0	0.125	4.0	32.0
2597	2.0	>256	16.0	32.0	16.0	16.0	0.50	0.064	64.0	0.25	0.50	64.0
4349	>256	16.0	4.0	8.0	1.0	8.0	0.25	0.064	2.0	0.125	2.0	8.0
3590	2.0	>256	32.0	64.0	32.0	128.0	1.0	0.064	8.0	1.0	8.0	64.0
2759	128.0	128.0	64.0	32.0	128.0	>256	2.0	0.125	64.0	4.0	1.0	16.0
3150	64.0	>256	8.0	8.0	2.0	8.0	0.50	0.25	8.0	0.25	0.50	8.0
286	8.0	>256	64.0	64.0	128.0	>256	1.0	0.064	16.0	>256	1.0	64.0
1168	2.0	>256	8.0	32.0	16.0	16.0	0.25	0.25	1.0	4.0	2.0	32.0
1288	0.25	>256	4.0	4.0	1.0	0.5	0.125	0.25	2.0	0.125	1.0	8.0
Isolate	TZ	TLC	PP	TPZ	AT	CR	PM	MP	AK	CL	IMI	TGC
2237	0.5	64.0	2.0	4.0	2.0	4.0	0.25	0.064	2.0	0.125	2.0	64.0
1104	1.0	256.0	64.0	32.0	4.0	4.0	0.50	0.25	2.0	8.0	2.0	32.0
2273	2.0	128.0	256.0	64.0	64.0	256.0	1.0	0.125	2.0	>256	16.0	32.0
2913	1.0	128.0	128.0	32.0	4.0	32.0	0.25	0.125	2.0	32.0	4.0	16.0
1834	>256	>256	32.0	128.0	32.0	64.0	0.50	0.50	2.0	>256	1.0	32.0
1885	1.0	>256	128.0	>256	256.0	>256	0.50	1.0	4.0	>256	0.50	16.0
3787	128.0	1.0	32.0	8.0	16.0	1.0	0.125	0.25	0.50	0.032	0.50	0.50
2711	4.0	>256	256.0	256.0	>256	>256	2.0	>256	8.0	8.0	>256	32.0
399	0.50	32.0	8.0	8.0	4.0	4.0	0.25	0.125	2.0	0.125	2.0	16.0
564	0.50	32.0	8.0	8.0	8.0	64.0	0.25	0.125	2.0	0.64	2.0	32.0
1444	1.0	>256	64.0	256.0	32.0	64.0	0.50	0.125	2.0	>256	1.0	64.0
1897	1.0	128.0	8.0	8.0	8.0	4.0	0.50	0.064	8.0	0.25	0.50	32.0
2971	>256	>256	>256	>256	>256	>256	8.0	0.125	2.0	0.125	1.0	16.0

390	2.0	>256	>256	16.0	16.0	>256	1.0	1.0	8.0	0.50	2.0	32.0
661	0.50	256.0	8.0	32.0	8.0	16.0	0.25	0.25	2.0	0.50	1.0	32.0
K1	0.50	32.0	4.0	2.0	2.0	4.0	0.25	0.125	2.0	0.125	1.0	16.0
K2	1.0	32.0	4.0	4.0	1.0	8.0	0.25	0.125	2.0	0.125	0.25	64.0
K3	2.0	>256	>256	>256	128.0	>256	2.0	32.0	32.0	>256	>256	128.0
K4	0.50	64.0	2.0	2.0	4.0	2.0	0.25	0.125	2.0	0.25	2.0	16.0
K5	2.0	128.0	256.0	64.0	64.0	32.0	0.50	0.064	4.0	2.0	2.0	64.0
K6	1.0	16.0	4.0	8.0	4.0	8.0	0.25	0.125	2.0	8.0	1.0	32.0
K7	1.0	64.0	8.0	8.0	8.0	16.0	4.0	0.125	8.0	0.25	2.0	16.0
K8	16.0	64.0	32.0	32.0	0.25	2.0	1.0	0.064	4.0	8.0	>256	128.0
K9	4.0	>256	32.0	64.0	>256	32.0	0.50	1.0	4.0	1.0	0.50	32.0
K10	2.0	>256	>256	32.0	32.0	>256	1.0	1.0	8.0	0.25	2.0	32.0
K11	>256	>256	>256	>256	64.0	>256	1.0	4.0	4.0	0.125	4.0	32.0
K12	32.0	>256	>256	>256	>256	>256	2.0	>256	64.0	>256	4.0	16.0
952	0.50	48.0	8.0	8.0	4.0	8.0	0.25	0.125	4.0	0.64	4.0	16.0
1038	0.50	48.0	8.0	8.0	4.0	8.0	0.25	0.125	4.0	0.64	4.0	16.0
1337	2.0	8.0	4.0	4.0	8.0	0.50	1.0	0.50	2.0	16.0	1.0	32.0
1275.3	>256	>256	256.0	128.0	8.0	>256	2.0	0.50	128.0	>256	2.0	32.0
1040	32.0	>256	>256	>256	256.0	>256	1.0	16.0	4.0	0.125	4.0	16.0
2777	0.50	>256	32.0	32.0	64.0	16.0	0.25	0.064	2.0	0.25	1.0	32.0
2951	1.0	>256	>256	128.0	64.0	32.0	0.50	0.25	2.0	2.0	1.0	32.0
3079	0.50	16.0	2.0	4.0	4.0	32.0	0.125	0.064	2.0	0.50	4.0	32.0
3084	4.0	>256	>256	>256	>256	>256	32.0	>256	>256	>256	>256	64.0
3121	0.50	32.0	4.0	2.0	4.0	16.0	0.25	0.50	2.0	0.125	1.0	16.0
3228	1.0	>256	64.0	128.0	256.0	128.0	0.50	>256	2.0	>256	16.0	32.0
2241	>256	>256	>256	>256	>256	>256	8.0	8.0	32.0	>256	4.0	64.0
2243	1.0	128.0	16.0	16.0	32.0	>256	1.0	0.25	8.0	2.0	2.0	64.0
1871	1.0	32.0	2.0	4.0	2.0	>256	1.0	0.125	32.0	>256	1.0	64.0
1092	>256	>256	>256	128.0	>256	>256	4.0	0.50	64.0	>256	2.0	32.0
127C	>256	>256	>256	128.0	>256	>256	4.0	0.50	64.0	>256	2.0	32.0
3362	0.50	32.0	4.0	32.0	4.0	16.0	0.25	0.25	8.0	0.25	2.0	32.0
3420	0.50	32.0	4.0	32.0	4.0	16.0	0.25	0.125	4.0	0.125	2.0	32.0

Isolate	TZ	TLC	PP	TPZ	AT	CR	PM	MP	AK	CL	IMI	TGC
3360	1.0	32.0	4.0	8.0	4.0	16.0	0.50	0.064	2.0	0.25	4.0	32.0
3504	1.0	>256	4.0	32.0	8.0	16.0	0.5	2.0	2.0	0.125	2.0	64.0
3583	>256	>256	>256	>256	>256	>256	128.0	>256	>256	>256	>256	32.0
1126	1.0	32.0	4.0	4.0	2.0	8.0	0.25	0.125	4.0	0.25	2.0	64.0
4631	1.0	32.0	4.0	4.0	2.0	8.0	0.25	0.125	4.0	0.25	2.0	64.0
4201	128.0	>256	>256	>256	>256	>256	2.0	0.25	8.0	32.0	2.0	32.0
4329	1.0	>256	32.0	32.0	64.0	64.0	0.25	0.125	2.0	32.0	2.0	128.0
4357	0.75	>256	16.0	32.0	32.0	32.0	0.25	0.125	2.0	2.0	2.0	16.0
4685	64.0	>256	>256	>256	256.0	>256	2.0	1.0	4.0	4.0	1.0	8.0
4629	>256	>256	>256	>256	>256	>256	8.0	4.0	4.0	2.0	32.0	8.0
4519	0.25	2.0	0.50	0.25	0.125	0.064	0.125	0.064	2.0	0.064	1.0	4.0

4696	>256	>256	>256	>256	>256	>256	8.0	2.0	32.0	>256	2.0	32.0
4730	1.0	32.0	>256	>256	>256	>256	2.0	>256	4.0	>256	>256	4.0
4710	>256	>256	>256	>256	>256	>256	64.0	>256	>256	>256	>256	32.0
4759	>256	>256	>256	>256	>256	>256	2.0	2.0	64.0	>256	1.0	32.0
5037	4.0	>256	32.0	>256	64.0	256.0	0.50	0.25	4.0	>256	1.0	64.0
5062	1.0	128.0	8.0	16.0	4.0	>256	0.50	0.25	2.0	0.50	0.50	32.0
5012	1.0	64.0	4.0	8.0	2.0	16.0	0.50	0.25	4.0	0.125	2.0	8.0
5197	0.50	32.0	4.0	8.0	8.0	8.0	0.25	0.064	4.0	0.125	2.0	16.0
5193	1.0	64.0	4.0	8.0	2.0	32.0	0.25	0.125	8.0	0.25	4.0	32.0
5103	1.0	256.0	16.0	32.0	8.0	>256	0.50	32.0	16.0	>256	>256	32.0
5102	32.0	16.0	>256	128.0	>256	>256	4.0	0.25	16.0	2.0	4.0	1.0
5198	1.0	>256	32.0	32.0	16.0	>256	0.50	32.0	16.0	>256	>256	>256
5199	0.50	32.0	4.0	4.0	8.0	16.0	0.25	0.064	4.0	0.125	2.0	16.0
5360	0.50	32.0	16.0	32.0	16.0	4.0	0.50	0.064	8.0	0.125	2.0	4.0
5785	0.5	>256	4.0	8.0	2.0	8.0	0.25	0.125	4.0	0.25	2.0	32.0

While two of the *Pseudomonas aeruginosa* strains isolated from diabetic foot infections were resistant to all tested antibiotics, all isolates except one were found to be intermediate or resistant to at least one antibiotic. The highest resistance rates among *P. aeruginosa* isolates were, Ticarcillin and Clavulanic acid (60%) respectively, It was determined as Ciprofloxacin (48%) and Aztreonam (45%). The highest sensitivity rates were found for the antibiotics cefepime (96%), meropenem (83%) and amikacin (81%), respectively (Table 3). Table 4 shows the MIC50, MIC90, range values of the antibiotics evaluated in our study (Table 4).

Table 3. Antibiotic sensitivity/resistance rates according to MIC results

Antibiotic	Rate of sensitive isolates (%)	Rate of intermediate isolates (%)	Rate of resistant isolates (%)
TZ	76	2	22
AT	49	6	45
MP	83	2	15
TGC	*There are no breakpoint values for P. aeruginosa*		
TLC	9	31	60
TPZ	45	27	28
AK	81	6	13
PP	52	18	30
PM	96	0	4
CL	4	48	48
IMI	73	12	15
CR	*There are no breakpoint values for P. aeruginosa*		

Table 4: MIC50, MIC90 and range values of the antibiotics evaluated in our study

Antibiotic	MIC50 (mg/mL)	MIC90 (mg/mL)	Range
TZ	1	>256	0,25- >256
AT	16	>256	0,125- >256
MP	0,125	32	0,032- >32
TGC	32	128	0,5- >256
TLC	256	>256	1- >256
TPZ	32	>256	0,25- >256
AK	4	64	0,5- >256
PP	16	>256	0,5- >256
PM	0,5	4	0,125- >256
CL	1	>32	0,032- >32
IMI	2	>32	0,25- >32
CR	32	>256	0,064- >256

DISCUSSION

Diabetes, with its rising prevalence and incidence, is considered as a global health problem, currently affecting around 171 million people worldwide and projected to reach 366 million by 2030 (Pedras et al.,2016). Diabetic patients have a higher rate of developing foot wound infections than non-diabetic individuals due to reasons such as deterioration of microvascular circulation, neuropathy, deterioration in immune capacity and anatomical changes. Therefore, one of the important complications of diabetes mellitus is diabetic foot ulcers (Abdulrazak et al.,2005). Diabetic neuropathy and peripheral arterial disease (PAD) play an important role in the development of foot ulcers complicated by infection and are also considered a strong indicator of amputation. Additionally, the high recurrence rate of diabetic foot ulcers, due to immune suppression and treatment costs ranging from $900 to $4,595 per episode in different countries, not only hampers the quality of life but also imposes a significant financial burden on patients (Mairghani et al.,2019).

It has been reported that over 50% of people with diabetic foot ulcers become infected by various types of microorganisms due to the damage to the protective skin layer. (Lipsky et al.,2012). Potential causative organisms of diabetic foot ulcers (DFUs) are believed to include *Staphylococcus, Streptococcus, Proteobacteria, Pseudomonas aeruginosa,* and coliform bacteria (Lipsky et al.,2015). Numerous studies have shown that *Staphylococcus aureus* is the predominant pathogen of diabetic foot ulcers in Western countries, while *Pseudomonas* is more common in Asian and African countries (Kwon and Armstrong,2018). Empiric antibiotic therapy is a crucial aspect of managing diabetic foot ulcers (DFUs). In recent years, the use of antimicrobial agents has become challenging due to the emergence of multi-drug resistant (MDR) bacteria, making the selection of appropriate antibiotics difficult. Antibiotic resistance rates have been increasing at an alarming rate in low- and middle-income countries. The rate of diabetic foot ulcers is increasing in developing countries due to inadequate knowledge about foot care among diabetic patients, an inefficient primary health care system, and low socio-economic status (Al-Rubeaan et al., 2015, Sharoni et al., 2017)

Aminoglycosides have a concentration-dependent bactericidal effect and are among the oldest antibiotics used in the treatment of infections caused by Gram-negative and some Gram-positive bacteria. While the amikacin resistance rate in *P. aeruginosa* varies between 0-29.5% in national studies, this rate is between 13.5-57% in international studies (-Kara et al.,2014, Du

et al.,2022, Öner et al.,2022, Makeri et al.,2023)-. The 13% resistance rate we obtained in our study is within the Türkiye and world average.
Ceftazidime, a third-generation cephalosporin, and cefepime, a fourth-generation cephalosporin, are antimicrobials with antipseudomal activity. In national studies, the cefepime resistance rate was reported as 24.2-42.3% and the ceftazidime resistance rate was reported as 11- 58,3 % -(Aykan and Çiftçi, 2015, Avcıoğlu et al.,2019, Erdoğan et al.,2021). When *the P. aeruginosa* resistance data of the SENTRY antimicrobial surveillance program, which includes the Asia-Pacific region, Europe, Latin America and North America, was evaluated between 1997 and 2016, the cefepime resistance rate was reported as 20.7% and the ceftazidime resistance rate was reported as 22.5% (Shortridge et al.,2021). In the meta-analysis study conducted by Du et al. in China, the ceftazidime resistance rate was 33.9%, the cefepime resistance rate was 20% (Du et al., 2022). In meta-analysis studies conducted in Africa, resistance rates were found to be 48.48% for ceftazidime and 22% for cefepime (Makeri et al., 2023, Wada et al., 2023). Cefpirome, a broad-spectrum cephalosporin tested in our study, does not have a breakpoint value for *Pseudomonas aeruginosa* in CLSI data. *Pseudomonas aeruginosa* can develop resistance mechanisms to various antibiotics, especially to broad-spectrum cephalosporins such as cefpirome.

Ciprofloxacin, a member of the fluoroquinolone class of antibiotics, has been used successfully to treat a wide range of bacterial infections since 1987 and is included in the World Health Organization's list of essential medicines (Rehman et al.2018). Ciprofloxacin allows oral or intravenously, and recently an inhalable treatment of many infections that require parenteral treatment due to its bioavailability, good penetration into tissue, and low side effects (Şenol,2002, Kłodzińska et al.,2016). In national studies, the ciprofloxacin resistance rate has been reported as 7-48% (Gönüllü et. al., 2003, Gültekin et al.,2004). According to the results of meta-analysis conducted in China, Africa and Iran, ciprofloxacin resistance in *Pseudomonas aeruginosa* was determined as 37.5%, 24.58% and 19.6%, respectively. (Shahrakh et al.,2021, Du et al.,2022, Wada et al.,2023). In our study, the ciprofloxacin resistance rate was found to be 48%. The resistance rate was higher than those mentioned studies.
The increasing level of carbapenem (imipenem/meropenem) resistance in *Pseudomonas aeruginosa* is an important public health problem. Resistance significantly limits treatment options. Significant differences are observed in the percentages of carbapenem-resistant *P. aeruginosa* across the WHO European Region. While resistance development of 5% or less was detected in only two of the countries where Central Asian and European Surveillance of Antimicrobial Resistance data were obtained, carbapenem resistance was found to be over 50% in 14 countries. Again, according to these data, the carbapenem resistance rate in Turkey is between 25-50% (WEB1, 2024). In our study, the carbapenem resistance rate was found to be 15% for both imipenem and meropenem, which showed lower percentage of resistance in comparison to other studies in Turkey and in many other countries.
Piperacillin extends the spectrum of activity of ampicillin to include most strains of *Pseudomonas aeruginosa*, *Enterobacteriaceae* (non-β-lactamase producing), many *Bacteroides* species, and *Escherichia faecalis*. (Pasifici,2023). In a study conducted in our country, the resistance rate to piperacillin was reported as 42.8%. According to the results of the meta-analysis conducted in China, piperacillin resistance rate was found to be 23,1% (Du et al., 2022).
The production of β-lactamases is the most common mechanism by which Gram-negative bacteria develop resistance to β-lactam antibiotics. A successful method to circumvent the threat of plasmid-encoded β-lactamases is to combine penicillin with inhibitors of these enzymes. Ticarcillin-clavulanate and piperacillin-tazobactam have the broadest spectra of activity, including effectiveness against *Pseudomonas aeruginosa*. Although ticarcillin-

clavulanate and piperacillin-tazobactam have similar spectra of activity, they exhibit many differences. (Lister, 2000). In various studies conducted in our country, the resistance rate to ticarcillin clavulanate and piperacillin tazobactam in *Pseudomonas aeruginosa* was found to be approximately 38%. According to the results of the meta-analysis conducted in China, piperacillin resistance rate was found to be 15,4% (Du et al., 2022) Piperacillin/tazobactam, which is formed when combined with the β-lactamase inhibitor tazobactam, has a much broader antibacterial spectrum (Pasifici,2023).) In a study covering Western Europe, the piperacillin-tazobactam resistance rate was found to be 25,2%. In the same study, this resistance rate was found to be 88.9% in *Pseudomonas aeruginosa* with multidrug resistance (Karlowsky et al., 2023). In our study, the ticarcillin clavulanate resistance rate was found to be 60% and the piperacillin/tazobactam resistance rate was 28%. In our study, the resistance rate developing in piperacillin tazobactam was at a level similar to the studies conducted in Turkey and the world, while the resistance rate in ticarcillin clavulanate was found to be quite high.

Aztreonam is the first monobactam drug to enter antibacterial therapy. It is completely synthetic. It has a relatively narrow spectrum. It can be used instead of aminoglycosides in Gram (-) bacterial infections. It is an antibiotic that has not been used in the treatment of *Pseudomonas aeruginosa* in our country in recent years. The *Pseudomonas aeruginosa* aztreonam resistance rate in our country was found to be 49.6%. In a study covering France, Germany, Italy, Portugal, Spain and the United Kingdom, aztreonam resistance was found to be 19.1%, and in the examination of *Pseudomonas* with multidrug resistance in the same countries, the resistance rate was found to be 73.1% (Karlowsky et al., 2023). In meta-analysis studies conducted in China, the aztreonam resistance rate in *Pseudomonas* was found to be 45.2% (Du et. al.,2022). The 45% resistance rate we found in our study is within the Türkiye average. It is thought that the reason why aztreonam resistance is so common may be due to other antibiotic groups that can induce the synthesis of plasmid-controlled extended-spectrum β-lactamase (ESBL) and chromosomal inducible β-lactamase (IBL) seen in *Pseudomonas* species and are in active use in treatment (Baddal et.al.,2021).

Studies have shown that the majority of *Pseudomonas aeruginosa* strains exhibit natural resistance to tigecycline, making its use as a monotherapy in treating *Pseudomonas* infections limited (Zhanel et al., 2008). In another study conducted by Biedenbach and colleagues, the activity of tigecycline against pathogens isolated from patients with skin infections was examined, and it was found that *Pseudomonas aeruginosa* isolates exhibited high resistance to tigecycline (Biedenbach et al.,2005). In our study, evaluation could not be performed for tigecycline against *Pseudomonas aeruginosa* isolates due to the absence of a breakpoint value in the CLSI data. Among the 100 isolates evaluated, a MIC value of 256 or higher was detected in 52 isolates.

The resistance rates we observed in our study are generally consistent with those found in other similar studies conducted in our country. However, contrary to the general situation, we found a higher sensitivity to carbapenems and a significantly lower resistance to cefepime. Differences in antibiotic susceptibility results among hospitals vary depending on antibiotic use policies, the clinic where the patients from whom the strains were isolated were hospitalized, and the patients' underlying diseases. The observed differences in antibiotic resistance patterns highlight the need for ongoing surveillance and collection of localized resistance data to guide effective treatment strategies.

REFERENCES

Abdulrazak A, Bitar ZI, Al-Shamali AA, Mobasher LA. Bacteriological study of diabetic foot infections. J Diabetes Complicat 2005;19(3):138-41.

Al Benwan K, Al Mulla A, Rotimi VO. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J Infect Public Health 2012;5(1):1-8.

Avcıoğlu F, Karabörk Ş, Kurtoğlu MG, Behçet M. Çeşitli klinik örneklerden izole edilen Pseudomonas aeruginosa suşlarının antimikrobiyal direnç oranları: üç yıllık değerlendirme. ANKEM Derg. 2019;33(2):43-8.

Aykan ŞB, Çiftci İH. Meta-Analiz: Türkiye'de Pseudomonas aeruginosa izolatlarının son 11 yıldaki antibiyotik direnç değişimi. Mikrobiyol Bul. 2015;49(3):352-65.

Baddal B., Güler E., Süer K. Hastane ve Toplum Kökenli İnfeksiyonlardan İzole Edilen Pseudomonas aeruginosa Suşlarında Antibiyotik Direnci: Kuzey Kıbrıs'ta Güncel Durum. Klimik Dergisi 2021; 34:31-6

Biedenbach DJ, Beach ML, Jones RN. Activity of Tigecycline and Comparators against Pathogens Isolated from Patients with Skin and Skin-Structure Infections. Diagnostic Microbiology and Infectious Disease.2005

Çakmaklıoğulları EK, Kuru C. Pseudomonas aeruginosa suşlarının antibiyotik duyarlılıkları: Farklı örnek türlerinde değerlendirme. ANKEM Derg. 2019;33(2):37-42.

Du F, Ma J, Gong H, Bista R, Zha P, Ren Y, Gao Y, Chen D, Ran X and Wang C (2022) Microbial Infection and Antibiotic Susceptibility of Diabetic Foot Ulcer in China: Literature Review. Front. Endocrinol. 13:881659. doi: 10.3389/fendo.2022.881659

Erdoğan MM, Acun Delen L, Erdoğan E. Yoğun bakım ünitelerinden izole edilen Pseudomonas aeruginosa suşlarının antibiyotiklere duyarlılıkları. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksek Okulu Derg. 2021;9(1):230-37.

Ertuğrul, M.B., Baktiroglu, S., Salman, S., Unal, S., Aksoy, M., Berberoglu, K., Calangu, S. 2008. "Pathogens isolated from deep soft tissue and bone in patients with diabetic foot infections", Journal of American Pediatric Medicine Association 98 (4), 290-295.

Ertuğrul, B,M,, Oncul, O., Tulek, N., Willke, A., Sacar, S., Tunccan, O.G., Yilmaz E., Kaya, O., Ozturk, B., Turhan, O. et al. 2012. "A prospective, multi-center study: factors related to the management of diabetic foot infections", European Journal of Clinical Microbiology and Infectious Diseases, 31 (9), 2345-2352.

Ertuğrul, M.B., Uyar-Güleç, G., Baktıroğlu, S., Çörekli E., Türe, M. 2017. "Diyabetik ayak infeksiyonu etkenlerinin yıllara göre dağılımı: Değişim var mı? Klimik Dergisi, 30, 27 - 31.

Gönüllü N, Gürol Y, Bülüç M, Bal Ç. Pseudomonas aeruginosa suşlarında görülen beta-laktam direnç fenotipleri ve antibiyotik duyarlılıkları. Hastane İnfeksiyonları Dergisi 2003; 7: 141-7.

Gültekin B, Eyigör M, Aydın N. Klinik örneklerden izole edilen Pseudomonas kökenlerinin antibiyotik direnci. ANKEM Derg 2004; 18 : 1-4.

Hatipoglu, M., Mutluoglu, M., Uzun, G., Karabacak, E., Turhan, V., Lipsky, B.A. 2014. "The microbiologic profile of diabetic foot infections in Turkey: a 20-year systematic review: diabetic foot infections in Turkey", European Journal of Clinical Microbiolology and Infectous Diseases, 33 (6), 871-878.

Iraj B., Khorvash F., Ebneshahidi A., Askari G. Prevention of diabetic foot ulcer. Int J Prev Med. 2013 Mar;4(3):373-6.

Jneid J., Cassir N., Schuldiner S., Jourdan N., Sotto A., Lavigne J.P. and La Scola B. Exploring the Microbiota of Diabetic Foot Infections With Culturomics. Front. Cell. Infect. Microbiol. 2018, 8:282. doi: 10.3389/fcimb.2018.00282

Joyce L.F., Downes J., Stockman K., and Andrew J H. Comparison of five methods, including the PDM Epsilometer test (E test), for antimicrobial susceptibility testing of Pseudomonas aeruginosa. J Clin Microbiol. 1992 Oct; 30(10): 2709–2713.

Kara Z.,Örmen B. Türker N., Vardar i., Ural S., El S., Kaptan F., Demirdal F. Diyabetik Ayak İnfeksiyonlarının Klinik ve Bakteriyolojik Olarak Değerlendirilmesi. Klimik Dergisi 2014; 27(1): 21-5

Karlowsky J.A., Loba SH, Siddiquic F., Akrichd, B., DeRyke CA., Young K., Motyl MR., Hawser SP., Sahm DF. In vitro activity of ceftolozane/tazobactam against multidrug-resistant Pseudomonas aeruginosa from patients in Western Europe: SMART 2017-2020. International Journal of Antimicrobial Agents 61 (2023) 106772

Khalid Al-Rubeaan ,Mohammad Al Derwish,Samir Ouizi,Amira M. Youssef,Shazia N. Subhani, Ibrahim HM., Alamri BN. Diabetic foot complications and their risk factors from an large retrospective cohort study. PLoS One 2015;10(5):e0124446.

Kłodzińska SN, Priemel PA, Rades T, Mørck Nielsen H. Inhalable antimicrobials for treatment of bacterial biofilm-associated sinusitis in cystic fibrosis patients: challenges and drug delivery approaches. Int J Mol Sci 2016; 17:1688

Kwon KT. and Armstrong DG. Microbiology and antimicrobial therapy for diabetic foot infections. Infection & chemotherapy 2018;50(1):11

Lipsky BA, Berendt AR, Cornia PB., Pile JC, Peters EJG., Armstrong DG., Deery HG, Embil JM., Joseph WS., Karchmer AW., Pinzur MS., Senneville E. Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. 2012 Clin Infect Dis 2012;54(12):e132e73.

Lipsky BA., Aragón-Sánchez J.,Diggle M., Embil J., Kono S., Lavery L.,Senneville E., Urbančič-Rovan V., Van-Asten S., Peters EJG., International Working Group on the Diabetic Foot. IWGDF guidance on the diagnosis and management of foot infections in persons with diabetes. Diabetes Metab Res Rev 2016;32(Suppl1):45e74.

Lister PD. β-Lactamase Inhibitor Combinations with Extended-Spectrum Penicillins: Factors Influencing Antibacterial Activity against Enterobacteriaceae and Pseudomonas aeruginosa. Pharmacotherapy 2000;20(9 Pt 2):213S–218S

Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.

Mairghani M, Jassim G., Elmusharaf K.,Patton D., Eltahir O., Moore Z., Sorensen J. Methodological approaches for assessing the cost of diabetic foot ulcers: a systematic literature review. J Wound Care 2019;28(5):261e6.

Makeri D. , Odoki M., Eilu E. 1 and Agwu E. Update on prevalence and antimicrobial resistance of Staphylococcus aureus and Pseudomonas aeruginosa isolated from diabetic foot ulcers in Africa: a systematic review and meta-analysis Bulletin of the National Research Centre (2023) 47:145https://doi.org/10.1186/s42269-023-01119-5

Öner SZ., Kaleli İ., Demir M., Mete E., Çalışkan A., Ergin Ç. PSEUDOMONAS AERUGINOSA İZOLATLARININ ANTİBİYOTİK DİRENCİ VE YILLAR İÇİNDEKİ DEĞİŞİMİ ANKEM Derg 2022;36(1):9-15. doi: 10.54962/ankemderg.1107814

Pacifici GM. Clinical Pharmacology of Piperacillin/Tazobactam. J Clin Biomed Invest 2023, 3:1

Pedras S, Carvalho R, Pereira MG. Quality of life in Portuguese patients with diabetic foot ulcer before and after an amputation surgery. Int J Behav Med 2016;23:714e21.

Ramakant, P., Verma, A.K, Misra, R., Prasad, K.N., Chand, G., Mishra, A., Agarwal, G., Agarwal, A., Mishra, S.K., 2011. "Changing microbiological profile of pathogenic bacteria in diabetic foot infections: time for a rethink on which empirical therapy to choose?" Diabetologia, 54 (1),58-64.

Sharoni SKA., Abdul Rahman H., Minhat HS:, Ghazali SS., Ong MHA.. A self-efficacy education programme on foot self-care behaviour among older patients with diabetes in a public long-term care institution, Malaysia: a Quasi-experimental Pilot Study. BMJ Open 2017;7(6):

e014393.
Shortridge D, Gales AC, Streit JM, Huband MD, Tsakris A, Jones RN. Geographic and temporal patterns of antimicrobial resistance in Pseudomonas aeruginosa over 20 years from the SENTRY antimicrobial surveillance program, 1997–2016. In Open Forum Infectious Diseases, 6. baskı, No. 1, s.63-8, Oxford University, US (2019).
Şenol E. Siprofloksasin. ANKEM Derg. 2002;16(3):382-84.
Wada F.W, Mekonnen M.F., Sawiso E.D.,Kolato S., Woldegiorgis L., Kera G.K., El-Khatib Z. Ashuro A.A.1, Biru M.and Boltena M.T. Bacterial profile and antimicrobial resistance patterns of infected diabetic foot ulcers in sub-Saharan Africa: a systematic review and meta-analysis. Scientific Reports | (2023) 13:14655 | https://doi.org/10.1038/s41598-023-41882-z
WEB1. https://www.who.int/europe/publications/i/item/9789289058537 Antimicrobial resistance surveillance in Europe 2023–2021 data. Erişim Tarihi: 28/06/2024
Zhanel GG, DeCorby M, Laing N, et al. In Vitro Activity of Tigecycline against Multidrug-Resistant Pseudomonas aeruginosa. Journal of Antimicrobial Chemotherapy. 2008

CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF WILD-GROWN *ORIGANUM VULGARE* L. SUBSP. *VULGARE* AND *THYMUS CAPITATUS* ESSENTIAL OILS FROM ALBANIA

ELTON BASHA [1,*], ERJON MAMOÇI [2], ANIKET SHARMA[3], ENTELA HODAJ-ÇELIKU[4], BLEDAR BISHA[3]

[1] Department of Agri-Food Technology, Agricultural University of Tirana, Tirana, Albania.
[2] Department of Food Science and Biotechnology, Agricultural University of Tirana, Tirana, Albania.
[3] Department of Animal Science, University of Wyoming, Wyoming, United States of America.
[4] Department of Chemistry, Agricultural University of Tirana, Tirana, Albania.
* Correspondence: ebasha@ubt.edu.al

The objective of this study was to chemically characterise and evaluate the antimicrobial activity of essential oils (EOs) from red oregano (*Origanum vulgare* L. subsp. *vulgare)* and thyme (*Thymus capitatus)* grown in the wild in Albania against foodborne pathogens and key spoilage microorganisms. The antimicrobial activity was tested against five bacteria (*Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Micrococcus luteus, Stenotrophomonas maltophilia)* and one yeast *(Candida albicans),* all sourced from the American Type Collection Cultures (ATTC). Plants were collected in the wild in 2 different geographic locations in Albania, subsequently air-dried, and their essential oils were extracted by hydrodistillation method. EO yields (% v/w) were 0.25% for oregano and 0.75% for thyme. The chemical compositions of the EOs were determined using Gas Chromatography-Mass Spectrometry (GC-MS). GS-MS analysis revealed that red oregano and thyme main components were gremacrene D (15.2%), carvacrol (12%), β-Caryophyllene (13.8%) and carvacrol (76.74%), p-cymene (6.4%), γ-terpinene (4.5%) respectively. The antimicrobial activity was performed evaluating the Minimal Inhibitory Concentration (MIC). Thyme EO was effective against all tested microorganisms, whereas red oregano did not show activity against *Salmonella enteritidis and Pseudomonas aeruginosa.* Both EOs were equally effective against *Micrococcus luteus and Candida albicans*, however, thyme EO showed stronger activity towards *Escherichia coli* and *Stenotrophomonas maltophilia.*

Keywords: *Origanum vulgare* L. subsp. *vulgare, Thymus capitatus, antimicrobial, MIC*

1. INTRODUCTION

A major global concern facing humanity today is the resistance of microbes, mostly clinically significant bacteria, to antimicrobials, especially antibiotics essential to human treatment. (McEwen, S.A. et al. 2018). Pathogenic bacteria that represent a serious threat to human health, which spread also through food, include those from the Enterobacteriaceae and Pseudomonadaceae families which are immune to most of antibiotics recommended for treatment (Chaudhary, A.S. 2016). On the other hand, although oxidation is essential to the life cycle of cells, oxidative stress is the primary factor in the genesis of numerous human diseases, including cancer, Alzheimer's disease, atherosclerosis, arthritis, and cardiovascular disorders (Es-safi, I. et al. 2009). In the meantime, artificial antioxidants that stabilize food, including butylated hydroxytoluene (BHT), have been linked to cancer (Ito, N. 1986). One of the most attractive strategies in addressing these issues is the utilization of medicinal plants, many of which are well known for their antibacterial and antioxidant effects.

Essential oils (EOs) are becoming more and more popular because of their therapeutic qualities, which are postive for human health and because they are generally regarded as safe (GRAS) by the US Agency for Food and Drug Products (Fancello, F. 2016). The volatile secondary metabolites that constitute the EOs are naturally occurring compounds. Their complex mixture, which includes hydrocarbons of monoterpenes and sesquiterpenes, their oxygenated derivatives (such as alcohols, aldehydes, ketones, and ethers), several derivatives of phenyl propane, phenols, and various volatile organic compounds, is what gives them their distinctive aroma (Viuda-Martos, M. et al. 2010, Hammer, K.A. et al. 2010). It is known that EOs exhibit a variety of biological properties, including insecticidal, antifungal, and antibacterial properties (Celiktas, O.Y. et al. 2007). EOs of some medicinal plants have been shown in several studies to possess antioxidant and antibacterial properties (Es-safi, I. etal 2021, Bouhaddouda, R. et al. 2016, El Bouzidi, L. 2013). As a result, differences in the chemical composition can affect the antibacterial and antioxidant properties of EOs (Gachkar, L. et al. 2003, Hazzit, M. et al. 2009). Therefore, to validate the use of EOs as preservatives in the food, pharmaceutical, and cosmetic industries, analysis of their chemical composition and assessment of their biological activity are required.

Origanum vulgare L. subsp. *vulgare* and *Thymus capitatus* (L.) Hoffmanns. & Link, both belonging to the Lamiaceae family, are two medicinal and aromatic plants that are extensively found in the wild across Albania. These species are commonly utilized for both medicinal and culinary purposes.

Red oregano (*Origanum vulgare* L. subsp. *vulgare*), one of the two subspecies of *Origanum vulgare* L. present in Albania, alongside White oregano (*Origanum vulgare* L. subsp. *hirtum*), is particularly noteworthy. In northern Albania, where it grows naturally on mountain slopes, red oregano is locally referred to as "Mountain Tea." This subspecies is especially prevalent in the central and northern regions of the country, thriving in a range of environments at altitudes between 400 and 1300 meters above sea level (Kadiasi et al., 2024).

Compared to the *hirtum* subspecies, red oregano is characterized by a lower essential oil content, with concentrations reaching up to 2%. This lower essential oil content makes red oregano particularly suitable for use in herbal teas, which are traditionally employed in the treatment of colds and sore throats (Pieroni, 2008).

The genus *Thymus* is considered to be one of the eight most important genera in the Lamiaceae family comprising around 215 species, which are native to the Mediterranean basin (Pirbalouti, A.G. et al. 2015). Thyme’s medicinal and aromatic properties have made it one of the most popular genera in the world. Besides, its essential oils are widely used to flavour and preserve several food products. Several studies linked *Thymus* EO chemical composition to its antimicrobial and antioxidant activities (Ballester-Costa, C. et al. 2017). *T. capitatus*, known also as *Thymbra capitata* (L.) Cav. or *Satureja capitata* (L.) Cav., is an endemic Mediterranean plant (Figueiredo, A. et al. 2008). In Albania it is predominantly found in the south of the country were often it is referred locally as “wild oregano”, due to the similar with the commonly used white oregano, probably because both have the same main components, carvacrol and thymol (Ibraliu A. et al. 2011).

The present study aimed to investigate the chemical composition and the antimicrobial activity of the EOs of two endemic plant species growing in Albania: *Origanum vulgare* subsp. *vulgare* and *Thymus capitatus* (L.) Hoffmanns. & Link.

2. MATERIALS AND METHODS

2.1 Plant material

Both plants were collected in July 2023. *Origanum vulgare* subsp. *vulgare* population was collected at the Mountain of Balgjajt, near Kurdari village, Albania, at coordinates 41°35'27" latitude (N); 20°08'35" longitude (E). *Thymus capitatus* (L.) Hoffmanns. & Link. was collected above Livadh beach, Himara, Albania at coordinates 40°06'38"latitude (N) 19°43'50" longitude (E). The species identification for each population was carried out by, and also herbarium specimen vouchers were deposited in, the Genetics and Plant Breeding Laboratory, Agricultural University of Tirana, Albania. The plant materials were dried in a well–ventilated, shaded area, at room temperature about 25 °C and relative humidity around 50%.

2.2 EO extraction

The essential oils were extracted by hydrodistillation using a Clevenger apparatus. One hundred grams of dried plant material, including of flowers, stems, and leaves, were minced finely and put into a one-liter flask with half a liter of distilled water. The distillation process was carried out over a period of three hours, with a steady distillation rate of three milliliters per minute. The essential oil yield was calculated as a percentage of volume by weight (% v/w) relative to the dry weight of the plant material. The obtained EOs were stored at 4 °C prior to analysis.

2.3 Microbial strains

The bacterial and fungal ATCC strains (American type culture collection strain (ATCC) *Salmonella enteritidis* (ATCC:49223), *Escherichia coli* (ATCC:10535), *Pseudomonas aeruginosa* (ATCC:9027), *Stenotrophomonas maltophilia* (ATCC:13637). *Micrococcus luteus* (ATCC:10240), along with one fungal isolate *Candida albicans* (ATCC:10231)) were procured from Microbiologics , Inc., (Minnesota United States). 96-well plates were secured from Corning Inc. (New York, United States).

Blood agar medium and Muller Hinton Broth were procured from Remel Inc, (California, United States) ,0.5 Polymer McFarland Standard from Thermo Fisher Scientific (Massachusetts, United States) and Dimethylsulfoxide (DMSO) from Sigma-Aldrich (Missouri, United States).

2.4 Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry Essential oil analyses were performed on a Shimadzu GC-2010-GCMSQP2010 system operating at 70 eV. The temperature program was from 60 ^{o}C to 250 ^{o}C, at a rate of 5 ^{o}C/min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Injection volume of each sample was 1 μL. Retention indices for all compounds were determined according to Van den Dool and Kratz, 1963, using n-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of

NIST21 and NIST107, Massada, 1976 and by comparison of their retention indices with literature data Adams, 2007. Component relative concentrations were calculated based on GC peak areas without using correction factors. Essential oils were often subjected to co-chromatography with authentic compounds (Fluka, Sigma).

2.5 Evaluation of antimicrobial activity by Micro-dilution Broth Experiment

Microbial strains and growth conditions: The Essential Oil (EO) antimicrobial activities were investigated against different clinical and food-borne pathogens using standard American type culture collection strain, five bacteria along with one fungal isolate. The bacterial and fungal ATCC strains were stored at 4°C and sub-cultured for the experimental setup. Prior to the inoculation of the strains with EOs, the microorganisms were grown at 28 or 37 °C (for fungus and for bacteria) for 18-20 h on blood agar medium.
Evaluation of MIC: The MIC (Minimum Inhibitory Concentration) of each EO was determined using a broth microdilution method in 96-well plate in accordance with the Clinical & Laboratory Standards Institute (CLSI) protocols (M100 Ed34, 2024). In brief, bacterial suspensions were adjusted to a final concentration of 10^5 CFU/mL cells standardized by 0.5 McFarland in Muller Hinton Broth (MHB) media. EO stock concentration of 100mg/ml was prepared by dissolving in DMSO. From this stock, a working concentration of 5mg/ml was prepared (in MHB media) to be used in plate susceptibility testing, ensuring DMSO conc. less than 5%. This resulted in EO's concentration range of 5mg/ml to 0.0097 mg/ml on the plate. One hundred microliters of bacterial suspension were finally added to each well. The plate setup included the 11^{th} column as the media control (negative control), and the 12th column containing bacteria and media (positive control). Additionally, rows D and E were designated for solvent controls, with row D with only EO (solvent control) and row E for DMSO (conc. used to dissolve EO) and bacteria served as DMSO control. The plates were incubated at 37 °C for 24 h. MIC was determined using Tecan I-control software (Infinite M Plex TECAN) to measure the OD (600nm) compared with positive control. Everything was kept constant for determining antifungal activity except incubation was done for 45-48h, with OD determined at 530nm. MIC was determined as the lowest concentration of EO that inhibited visible growth of the tested microorganism (Puškárová A et al. 2017).

3. RESULTS AND DISCUSSION

3.1 EO extraction

The essential oil of *Origanum vulgare* subsp. *vulgare* had a pale yellow color with a yield of 0.25% (v/w). This result is in line with another study of Albanian Red oregano where Kadiasi et al. (2024) reported that from 15 individuals from seven different red oregano accessions collected from various Albanian locations had an EO yield ranging from 0.05% to 1.3%. Similar results were reported for this species from other countries: 0.12% in serbia (Aćimović M. et al. 2020), 0-7-1.2% in Montenegro (Stešević, D. et al. 2018), 0.108% and 0.249% from Moldova (Vazirian, M. et al. 2014), 0.35 to 0.87% in Poland (Kosakowska, O. et al. 2018) and

0.08% in Türkiye (Sezik, E. et al. 1993). From this the population in study is positioned as with medium yield of EO.
The EO extracted from *Thymus* resulted in pale-yellow colour, with a yield of 0.21 % (v/w). The authors did not find any existing reports on the EO yield of *Thymus capitatus* from Albania. However, when compared with unpublished data, this particular population demonstrates a relatively low yield. In contrast, other populations of *T. capitatus* have been reported to yield approximately 1% EO.

3.1 Quantitative and qualitative analysis of the EO

GC-MS analysis of the *Origanum vulgare* subsp. *vulgare* EO (Fig. I) identified in total 20 different components, counting for 83% of the EO. The most abundant compounds in were gremacrene D (15.2%), β-Caryophyllene (13.8%), carvacrol (12%) and several other compounds like Eucalyptol, Terpinen-4-ol, γ-Eudesmol, p-Cymene, α-Farnesene ect (Table 1). This results are in line with other reports from Albania (Kadiasi, N. et al. 2018, Kadiasi, N. et al. 2024). Studies from other countries show a high variation of the main compounds where depending on environmental factors Sabinene, p-Cymene, 1,8-Cineole, γ -Terpinene, Linalool, Linalyl Acetate, Thymol, Carvacrol, α-Terpineol, β-Citronellol, Linalyl Acetate, Thymol, Carvacrol, β -Caryophyllene, Germacrene D, Caryophyllene Oxide interchange as principal components (Aćimović M. et al. 2020).

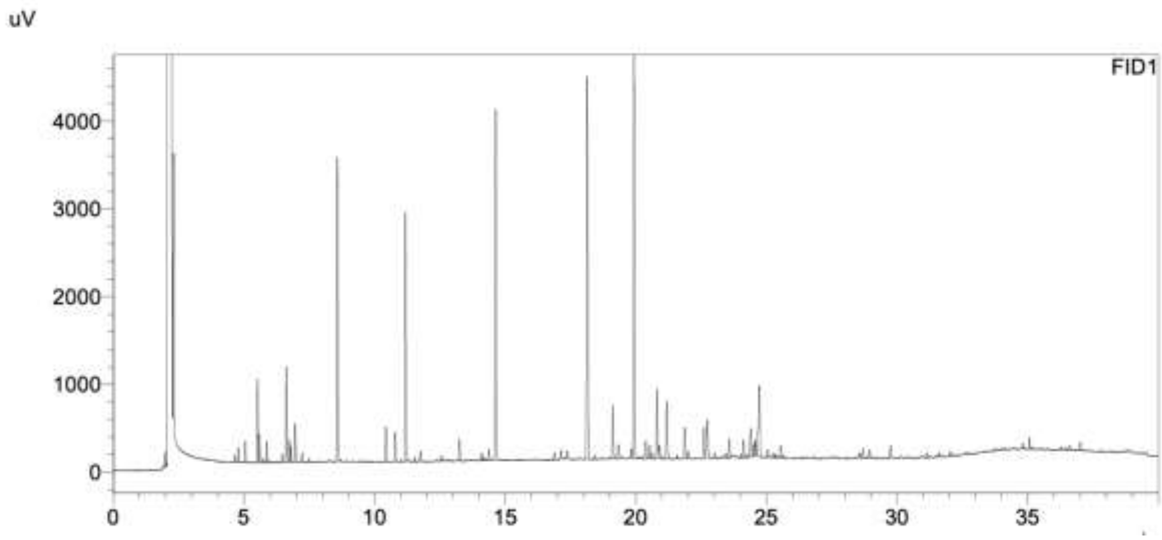


Figure. 1. GC-MS chromatogram of *Origanum vulgare* subsp. *vulgare* essential oil

Table 1. Composition of the essential oil from *Origanum vulgare* L. subsp.*vulgare*

Compounds [a]	**AI [b]**	**%**	**ID [c]**
α-Pinene	935	0.6	AI, MS, Co-GC
Sabinene	976	2.1	AI, MS
α-Phellandrene	1007	0.8	AI, MS
α-Terpinene	1019	0.6	AI, MS
p-Cymene	1028	2.6	AI, MS, Co-GC
Eucalyptol	1034	9.1	AI, MS
γ-Terpinene	1062	1.2	AI, MS, Co-GC
Bornyl acetate	1075	1.2	AI, MS
Terpinen-4-ol	1186	8.2	AI, MS, Co-GC
Thymol	1298	0.7	AI, MS, Co-GC
Carvacrol	1309	**12.0**	AI, MS
β-Caryophyllene	1419	**13.8**	AI, MS, Co-GC
γ -Muurolene	1487	1.9	AI, MS, Co-GC
α-Farnesene	1489	2.6	AI, MS
Vividiflorol	1495	2.3	AI, MS
γ-Cadinene	1514	1.4	AI, MS
δ -Cadinene	1524	1.7	AI, MS
Germacene D	1587	**15.2**	AI, MS, Co-GC
Caryophyllene oxide	1589	1.5	AI, MS, Co-GC
γ-Eudesmol	1610	3.5	AI, MS
Total (%)		**83**	

[a]Compounds listed in order of elution from an HP-5 MS capillary column; [b]AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23); [c]Identification method: AI=Arithmetic Index, MS=mass spectrum, Co-GC=Coinjection with authentic compound.

GC-MS analysis of the *Thymus capitatus* EO (Fig. 2) identified in total 18 different components, counting for 96.14% of the EO. The most abundant compounds in were carvacrol (76.74%), p-cymene (6.4%), γ-terpinene (4.5%). The results were in line with other reports where Ibraliu at al. (2011) reported 4 different populations of *T. capitatus* from Albania with a mean of 71.3% carvacrol, 6.6% p-cymene and 3.5% γ-terpinene (Table 2). Also other reports from the mediterranean area showed the same main compounds (Gitsopoulos T.K. et al. 2013; Tagnaout I. et al. 2022; Russo, M. et al 2013). This population can be classified as carvacrol chemotype (Ćavar Zeljković, S. et al. 2015).

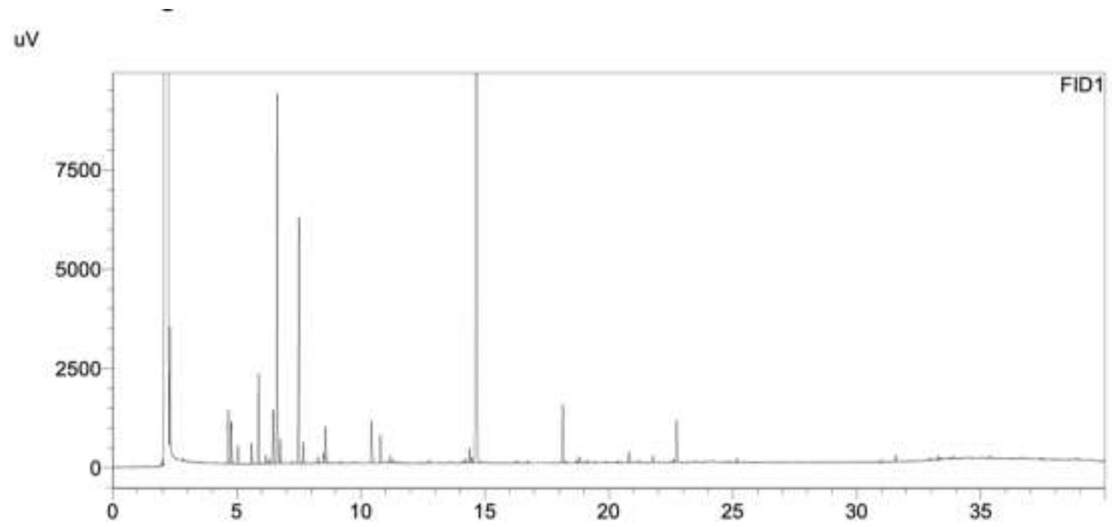


Figure 2. GC-MS chromatogram of Thymus capitatus essential oil

Table 2. Composition of the essential oil from *Thymus capitatus*

Compounds [a]	AI [b]	%	ID [c]
α-Pinene	931	1.4	AI, MS, Co-GC
β-Pinene	973	0.1	AI, MS, Co-GC
β-Myrcene	992	0.1	AI, MS, Co-GC
α-terpinene	931	0.9	AI, MS, Co-GC
β-Phellandrene	1003	0.4	AI, MS
p-Cymene	1024	**6.4**	AI, MS, Co-GC
γ-terpinene	1055	**4.5**	AI, MS, Co-GC
cis-Sabinenehydrate	1067	0.4	AI, MS
Linalool	1101	0.7	AI, MS, Co-GC
Borneol	1164	0.9	AI, MS, Co-GC
4-carvomenthenol	1185	0.6	AI, MS, Co-GC
o-cymen-5-ol	1280	0.2	AI, MS, Co-GC
2-isopropyl-5-methyl-phenol	1295	0.3	AI, MS, Co-GC
Carvacrol	1304	**76.74**	AI, MS
Caryophyllene	1419	1.3	AI, MS, Co-GC
Spathulenol	1578	0.2	AI, MS
Carryophyllene oxide	1583	1.0	AI, MS, Co-GC
Total		**96.14**	

[a]Compounds listed in order of elution from an HP-5 MS capillary column; [b]AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23); [c]Identification method: AI=Arithmetic Index, MS=mass spectrum, Co-GC=Coinjection with authentic compound.

3.3 Antimicrobial activity

Table 3 shows the antibacterial activity of essential oils from *Origanum vulgare* L. subsp. *vulgare* and *Thymus capitatus* as Minimum Inhibitory Concentration. Red oregano EO did not show any activity against *Salmonella enteritidis, Pseudomonas aeruginosa,* at the maximum tested concentration (5 mg/ml). It inhibited the growth of *E. coli* at 5 mg/ml, *Stenotrophomonas maltophilia* at 2.5 mg/ml, *Micrococcus luteus* at 1.25, and was most effective against the fungal strain *Candida albicans* with a MIC of 0.32 mg/ml.

Meanwhile *Thymus capitatus* EO was in general much more effective against the tested strains, showing antimicrobial activity against all of them. The lower effect was on *Pseudomonas aeruginosa* with a MIC of 2.5 mg/ml, and the highest effect against *Stenotrophomonas maltophilia* (MIC=0.15 mg/ml). MICs for *E. coli*, *Salmonella enteritidis, Micrococcus luteus* and *Candida albicans* were 0.31, 0.625, 1.25 and 0.31 mg/ml respectively.

The weak antibacterial activity of *O. vulgare* subsp. *vulgare* EO could be attributed to low concentrations of bioactive compounds and their inability to exhibit activity (Aćimović M. et al. 2020). *Thymus capitatus* was much more effective because its main compound, carvacrol is well reported for its remarkable antimicrobial properties (Baser K.H. 2008).

To our knowledge this is the first report that shows the antibacterial activity of essential oil from *Origanum vulgare* L. subsp. *vulgare* and *Thymus capitatus* EOs on *S. maltophilia*.

Table 3. Minimum Inhibitory Concentration of essential oil from *Origanum vulgare L. subsp.vulgare* and *Thymus capitatus* EOs.

Microorganism	**Minimum Inhibitory Concentration**	
	Origanum vulgare L. subsp.vulgare EO	*Thymus capitatus EO*
E. coli ATCC 10535	5 mg/ml	0.31 mg/ml
S. enteritidis ATCC 49223	NO MIC	0.625 mg/ml
P. aeruginosa ATCC 9027	NO MIC	2.5 mg/ml
M. luteus ATCC 10240	1.25 mg/ml	1.25 mg/ml
S. maltophilia ATCC 13637	2.5 mg/ml	0.15 mg/ml
C. albicans ATCC 10231	0.31 mg/ml	0.31 mg/ml

4. CONCLUSIONS

The purpose of this study was to analyze the chemical composition and antibacterial properties of the essential oils (EOs) derived from two populations of *Origanum vulgare* L. subsp. *vulgare* and *Thymus capitatus*, both of which are extensively used for culinary and medicinal purposes and grow wild in Albania.

Our findings corroborate a limited number of previous reports on the primary constituents of the EOs from these plants collected in Albania. Notably, while both EOs exhibited comparable efficacy against *Candida albicans*, *Thymus capitatus* demonstrated superior antimicrobial activity.

In conclusion, as the demand for new, natural, and safe antimicrobial agents grows across various sectors, the examination of the chemical profile and evaluation of the antibacterial activity of these species indicates their potential as valuable sources of natural agents. The outcomes of this investigation suggest that *Thymus capitatus* essential oil, in particular, could be effectively applied in the development of functional foods and as a pharmaceutical ingredient to enhance well-being.

Funding statement

This publication was made possible with the financial support of National Agency for Scientific Research, Technology and Innovation (NASRI), Albania. Its content is the responsibility of the author, the opinion expressed in it is not necessarily the opinion of NASRI.

5. REFERENCES

Aćimović M, Zorić M, Zheljazkov VD, Pezo L, Čabarkapa I, Stanković Jeremić J, Cvetković M. Chemical Characterization and Antibacterial Activity of Essential Oil of Medicinal Plants from Eastern Serbia. Molecules. 2020 Nov 23;25(22):5482.

Adams RP. (2007) Identification of essential oil components by gas chromatography/mass spectrometry. 4th ed., Allured Publishing Corporation, Carol Stream, IL.

Ballester-Costa, C.; Sendra, E.; Fernández-López, J.; Pérez-Álvarez, J.A.; Viuda-Martos, M. Assessment of Antioxidant and Antibacterial Properties on Meat Homogenates of Essential Oils Obtained from Four Thymus Species Achieved from Organic Growth. Foods 2017, 6, 59.

Baser KH. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. Curr Pharm Des. 2008;14(29):3106-19.

Bouhaddouda, R.; Aouadi, N.; Labiod, S. Evaluation of chemical composition and biological activities of essential oil and methanolic extract of Origanum vulgare L. ssp. glandulosum (DESF.) Ietswaart from Algeria. Int. J. Pharmacogn. Phytochem. Res. 2016, 8, 104–112.

Ćavar Zeljković, S., Maksimović, M. Chemical composition and bioactivity of essential oil from Thymus species in Balkan Peninsula. Phytochem Rev 14, 335–352 (2015).

Celiktas, O.Y.; Kocabas, E.E.H.; Bedir, E.; Sukan, F.V.; Ozek, T.; Baser, K.H.C. Antimicrobial activities of methanol extracts and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chem. 2007, 100, 553–559.

Chaudhary, A.S. A review of global initiatives to fight antibiotic resistance and recent antibiotics' discovery. Acta Pharm. Sin. B **2016**, 6, 552–556.

El Bouzidi, L.; Jamali, C.A.; Bekkouche, K.; Hassani, L.; Wohlmuth, H.; Leach, D.; Abbad, A. Chemical composition, antioxidant and antimicrobial activities of essential oils obtained from wild and cultivated Moroccan Thymus species. Ind. Crop. Prod. 2013, 43, 450–456.

Es-safi, I.; Mechchate, H.; Amaghnouje, A.; Elbouzidi, A.; Bouhrim, M.; Bencheikh, N.; Hano, C.; Bousta, D. Assessment of Antidepressant-Like, Anxiolytic Effects and Impact on Memory of Pimpinella Anisum L. Total Extract on Swiss Albino Mice. Plants 2021, 10, 1573.

Es-safi, I.; Mechchate, H.; Amaghnouje, A.; Kamaly, O.M.A.; Jawhari, F.Z.; Imtara, H.; Grafov, A.; Bousta, D. The Potential of Parsley Polyphenols and Their Antioxidant Capacity to Help in the Treatment of Depression and Anxiety: An In Vivo Subacute Study. Molecules 2021, 26, 2009.

Fancello, F.; Petretto, G.L.; Zara, S.; Sanna, M.L.; Addis, R.; Maldini, M.; Foddai, M.; Rourke, J.P.; Chessa, M.; Pintore, G. Chemical characterization, antioxidant capacity and antimicrobial activity against food related microor-ganisms of Citrus limon var. pompia leaf essential oil. LWT-Food Sci. Technol. 2016, 69, 579–585.

Figueiredo, A.; Barroso, J.; Pedro, L.; Salgueiro, L.; Miguel, M.; Faleiro, M. Portuguese Thymbra and Thymus Species Volatiles: Chemical Composition and Biological Activities. Curr. Pharm. Des. 2008, 14, 3120–3140.

Gachkar, L.; Yadegari, D.; Rezaei, M.; Taghizadeh, M.; Astaneh, S.D.; Rasooli, I. Chemical and biological characteristics of Cuminum cyminum and Rosmarinus officinalis essential oils. Food Chem. 2007, 102, 898–904.

Gitsopoulos TK, Chatzopoulou P, Georgoulas I (2013) Effects of essential oils of Lavandula x hybrida Rev., Foeniculum vulgare Mill. and Thymus capitatus L. on the germination and radical length of Triticum aestivum L., Hordeum vulgare L., Lolium rigidum L. and Phalaris brachystachys L. J Essent Oil Bear Plants 16(6):817–825

Genceariuc, M.; Balamus, Z.; Sandu, T.; Romanciuc, G.; Genceariuc, N. Essential oil of Origanum vulgare ssp. vulgare L. and Origanum vulgare ssp. hirtum (Link) Ietswaart from Moldova: Content and chemical composition. Int. J. Agric. Innov. Res. 2014, 3, 659–663.

Hammer, K.A.; Carson, C.F. Antibacterial and Antifungal Activities of Essential Oils. Lipids Essent. Oils Antimicrob. Agents **2010**, 6, 255–306.

Hazzit, M.; Baaliouamer, A.; Veríssimo, A.; Faleiro, M.L.; Miguel, M. Chemical composition and biological activities of Algerian Thymus oils. Food Chem. 2009, 116, 714–721.

Ibraliu, A.; Mi, X.; Risti´c, M.; Stefanovic, Z.D.; Shehu, J. Analysis of essential oils of three wild medicinal plants in Albania. J. Med. Plants Res. 2011, 5, 58–62.

Ito, N.; Hirose, M.; Fukushima, G.; Tauda, H.; Shira, T.; Tatematsu, M. Studies on activity of conventional antibiotics. Ind. Crop. Prod. 1986, 71, 37–43.

Kadiasi, N.; Hajdari, A.; Christian, I.; Tako, R.; Ibraliu, I. Variability of the Essential Oil Composition in Origanum vulgare subsp. vulgare Collected in Albania. Albanian J. Agric. Sci. 2018, 17, 91–97.

Kadiasi, N.; Tako, R.; Ibraliu, A.; Stanys, V.; Gruda, N.S. Principal Component and Hierarchical Cluster Analysis of Major Compound Variation in Essential Oil among Some Red Oregano Genotypes in Albania. *Agronomy* **2024**, *14*, 1419.

Kosakowska, O.; Czupa,W. Morphological and Chemical variability of common oregano (Origanum vulgare L. subsp. vulgare) occurring in eastern Poland. Herba Pol. 2018, 64, 11–21.

Kustrak D, Martinis Z, Kuftinec J, Blazevic N (1990) Composition of the essential oils of some Thymus and Thymbra species. Flavour Fragr J 5:227–231

Lukas, B.; Schmiderer, C.; Novak, J. Essential oil diversity of European Origanum vulgare L. (Lamiaceae). Phytochemistry 2015, 119, 32–40.

Massada Y. (1976) Analysis of essential oils by gas chromatography and mass spectrometry. John Wiley & Sons, New York.

McEwen, S.A.; Collignon, P.J. Antimicrobial resistance: A One Health perspective. Microbiol. Spectr. 2018, 6, 2.

Pieroni, A. Local plant resources in the ethnobotany of Theth, a village in the Northern Albanian Alps. Genet. Resour. Crop Evol. 2008, 55, 1197–1214.

Pirbalouti, A.G.; Bistghani, Z.E.; Malekpoor, F. An overview on genus Thymus. J. Herb. Drugs 2015, 6, 93–100.

Russo, M., Suraci, F., Postorino, S., Serra, D., Roccotelli, A., Agosteo, G.E., 2013. Essential oil chemical composition and antifungal effects on sclerotium cepivorum of Thymus capitatus wild populations from Calabria, Southern Italy. Revista Brasileira De Farmacognosia 23, 239–248.

Sezik, E.; Tumen, G.; Kirimer, N.; Ozek, T.; Baser, K.H.C. Essential oil composition of four Origanum vulgare subspecies of Anatolian origin. J. Essent. Oil Res. 1993, 5, 425–431.

Steševi´c, D.; Ja´cimovi´c, Ž.; Šatovi´c, Z.; Šapˇcanin, A.; Janˇcan, G.; Kosovi´c, M.; Damjanovi´c-Vratnica, B. Chemical characterization of wild growing Origanum vulgare populations in Montenegro. Nat. Prod. Commun.

Tagnaout I, Zerkani H, Hadi N, El Moumen B, El Makhoukhi F, Bouhrim M, Al-Salahi R, Nasr FA, Mechchate H, Zair T. Chemical Composition, Antioxidant and Antibacterial Activities of Thymus broussonetii Boiss and Thymus capitatus (L.) Hoffmann and Link Essential Oils. Plants (Basel). 2022 Mar 31;11(7):954.

Van den Dool H, Kratz PD. (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography.Journal of Chromatography A. 11, 463-471.

Vazirian, M.; Mohammadi, M.; Faraei, M.H.; Amin, G.; Amanzadeh, Y. Chemical composition and antioxidant activity of Origanum vulgare subsp. vulgare essential oil from Iran. Res. J. Pharmacogn. 2015, 2, 41–46.

Viuda-Martos, M.; El Gendy, A.E.-N.G.S.; Sendra, E.; Fernández-López, J.; El Razik, K.A.A.; Omer, E.A.; Pérez-Alvarez, J.A. Chemical Composition and Antioxidant and Anti-Listeria Activities of Essential Oils Obtained from Some Egyptian Plants. J. Agric. Food Chem. 2010, 58, 9063–9070.

World Health Organization, Antimicrobial resistance, WHO Media Center. Available online: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

DETERMINATION OF THE PHYTOCHEMICAL PROPERTIES OF MULBERRY (*MORUS* SPP.) SEED OIL AND ITS USAGE POTENTIAL IN THE FOOD INDUSTRY AND HUMAN HEALTH

Halil İbrahim OĞUZ[1] Leyla EREN KARAHAN[2]

[1]Adıyaman University, Faculty of Agriculture, Department of Horticulture
[2]Adıyaman University Faculty of Engineering, Department of Food Engineering

Corresponding author: hioguz@adiyaman.edu.tr

a

ABSTRACT

Mulberry is a berry known worldwide for its over 150 species, commonly represented by *Morus alba* L. (white mulberry), *Morus nigra* L. (black mulberry), and *Morus rubra* L. (red mulberry). The mulberry plant has a wide cultivation area, growing in both the northern hemisphere and tropical and subtropical climates. Mulberries are consumed in various forms depending on traditional methods, including fresh, dried, juice, concentrate, molasses, and fruit leather.
Mulberries offer numerous health benefits. They can used medicinally as anthelmintic, anti-anemic, anti-diabetic, odontalgic, expectorant, laxative, emetic, hypoglycemic, and for treating dysentery and oral lesions. Additionally, mulberry seed oil is rich in δ-tocopherol, providing high antioxidant capacity, and can be added to other edible vegetable oils to enhance nutritional value in dietary food products. The significant amounts of tocopherols and sterols in mulberry seed oil serve as antioxidants in diets and supplements. The essential oils in mulberry seeds are mainly composed of monoterpenes, alkanes, and aldehydes. With its L-limonene content, mulberry seeds are also utilized as by-products in the juice and wine industries. The oil content of mulberry seed is similar to that of high-oil sesame. Obtaining bioactive substances from seed waste in mulberry processing facilities is natural, low-cost, and straightforward. These mulberry wastes can be converted into high-value compounds for the production of food, pharmaceuticals, cosmetics, and other industrial products. This study aims to investigate the chemical properties of mulberry seed oil in detail and highlight its potential applications in the food and pharmaceutical industries. Mulberry seed oil is notable for its high antioxidant capacity and rich tocopherol content, making it valuable component in dietary supplements and food products. Furthermore, the using of mulberry seed oil in phytotherapeutic applications is significant for its health benefits. Converting the waste produced in mulberry seed processing facilities into bioactive substances at a low cost enables the production of high-value products for food, pharmaceuticals, cosmetics, and other industries. In this context, the study aims to demonstrate the multifaceted industrial and health potential of mulberry seed oil.

Keywords: Mulberry, mulberry seed, mulberry seed oil, fatty acids, tocopherols

INTRODUCTION

Mulberry, botanically classified under the *Morus* genus within the Moraceae family, comprises flowering plants. It is known that there are 68 species in the *Morus* genus, with hundreds of varieties under a single subspecies. However, most botanists generally recognize only 10 to 16 species (Das and Mukherjee, 1986; Liang et al., 2012). The most well-known mulberry species include *M. alba* L. (white mulberry), *M. nigra* L. (black mulberry), and *M. rubra* L. (red

mulberry). *M. alba* L. produces white and purple fruits that are sweet and low in acid, *M. nigra* L. yields very dark fruits that are sweet with a slight tartness, and *M. rubra* L. offers dark fruits that are sweet with a high dry matter content and low acidity (Özgen et al., 2009). Due to their tolerance to various climatic conditions, mulberry plants are widely cultivated in temperate, tropical, or subtropical regions across the Northern and Southern Hemispheres (Abbas et al., 2014; Abbas et al., 2016). The seeds are oval-shaped, with a micropillar and nearly flat surface, appearing in yellow or brown. The seed coat consists of two layers: a hard and brittle outer layer called the testa, and a thin, papery, slightly brownish inner layer known as the tegmen. Inside the seed coat, there is a kernel containing an outer endosperm and an inner embryo. The embryo comprises a primary axis (plumule and radicle) and two cotyledons. Mulberry seeds contain 25-35% yellow pigment oil (Barnes, 2008; Biasiolo, 2004; Vijayan et al., 2011).
Recent studies have increasingly focused on the oil content of various plant seeds due to their significance in nutrition and human health. Research has demonstrated that plant seeds are rich in fatty acids and possess strong antioxidant activity. Consequently, research on plant seed oils is rapidly expanding (Sbihi et al., 2018; Chrysochou et al., 2022). In this context, different plant seed oils, particularly those rich in linoleic acid and linolenic acid, are of particular interest due to their monounsaturated and polyunsaturated fatty acid content. Linoleic acid, an "essential fatty acid," cannot be synthesized by the human body and is considered an anti-carcinogenic substance. Symptoms of linoleic acid deficiency may include dry skin, hair loss, and poor wound healing (Argon et al., 2019). Due to these reasons, the oil content of seeds from various fruit types is of significant interest. For instance, mulberry seed oil is claimed to be a valuable dietary source rich in linoleic acid. However, it is relatively poor in linolenic acid. Low levels of linolenic acid are preferred in edible oils because high levels can lead to undesirable odours and tastes in the oil. Additionally, linolenic acid can easily oxidize due to its three double bonds in the hydrocarbon chain, reducing the stability and shelf life of oils rich in linolenic acid. Mulberry seed oil offers significant advantages in terms of human health and oil shelf life due to its low linolenic acid content (Yao et al., 2020). In this regard, several studies have been conducted on mulberry seed oils. Gecgel et al. (2011) examined the composition of black mulberry seed oil and identified that it is rich in fatty acids such as oleic acid, linoleic acid, palmitic acid, and linolenic acid. They reported that the average linoleic acid content exceeds 73%, indicating that mulberry seed oil is a good source of essential fatty acids. A subsequent study revealed that mulberry seed oil contains high levels of δ-tocopherol, thus possessing high antioxidant capacity (Yılmaz and Durmaz, 2015). Both Gecgel et al. (2011) and Yılmaz and Durmaz (2015) investigated the composition of mulberry seed oil and reported its strong antioxidant activity. Additionally, Chang et al. (2018) found that mulberry seed oils were preserved in an oil-water emulsion system using acid-hydrolyzed egg albumin. Li et al. (2020) prepared a lipid consisting of n-6/n-3 polyunsaturated fatty acids in a microfluidic enzyme reactor using mulberry seed oil and linolenic acid. However, they noted that further detailed research is needed to clarify the composition and properties of mulberry seed oils.
In a study conducted in China, various mulberry varieties (Nongsang 12, Nongsang 14, Qiangsang 1, Qiangsang 2, Fengtian, Guisang, and Dongsheng) were analyzed for their seeds' physical and biochemical properties. The study reported the total oil, phenolic and flavonoid acids, and antioxidant power of mulberry seeds, which were derived from 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Dongsheng, Fengtian, and Guisang varieties exhibited the highest antioxidant activities (FRAP, DPPH), and it was suggested that these varieties could produce high-quality oil for health care or functional food components (Yao et al., 2020).
A study conducted in Korea examined the oil content, fatty acids, phytosterols, and tocopherol compositions of *Morus alba* and *Cudrania tricuspidata* seeds. The study also assessed the polyphenolic compounds and biological activities of defatted seed residues. The oil contents of

Morus alba and *Cudrania tricuspidata* seeds were found to be 29.36% and 16.69%, respectively, while the methanol (MeOH) extracts of defatted *Morus alba* and *Cudrania tricuspidata* seed residues were 5.10% and 6.22%, respectively. The compositions of the seed oils for these two species were 81.4% and 74.37% linoleic acid, 5.75% and 11.39% oleic acid, 8.40% and 10.18% palmitic acid, 3.52% and 3.0% stearic acid, and trace amounts of linolenic and arachidic acids. *Morus alba* seeds were noted for their higher phytosterol (507.59 mg/100 g oil), tocopherol (99.64 mg/100 g oil), and total flavonoid (106.50 mg/100 g seed) contents compared to *Cudrania tricuspidata* seeds, which had a higher total polyphenol content. The MeOH extract of *Morus alba* seed residues showed higher antioxidant, anti-diabetic, and anti-melanogenic activities compared to *Cudrania tricuspidata* seed residues. The main polyphenolic components in the MeOH extract of *Morus alba* seed residues were identified as trans-resveratrol (9.62 mg/100 g), quercetin (54.83 mg/100 g), and 4-prenylmoracin (48.70 mg/100 g). These results indicate that *Morus alba* seeds are a better source of essential nutrients with antioxidant, anti-diabetic, and anti-melanogenic activities compared to *Cudrania tricuspidata* seeds (Kim et al., 2010).

Gecgel et al. (2011) investigated the physicochemical properties of mulberry (*Morus nigra* L.) seeds and seed oil obtained from domestic black mulberry in 2008 and 2009. They found that the seeds contained 27.5-33% crude oil, 20.2-22.5% crude protein, 3.5-6% ash, 42.4-46.6% carbohydrates, and 112.2-152.0 mg of total phenolic compounds per 100 grams. Additionally, they noted that mulberry seeds contain 20 different fatty acids, with proportions ranging from 0.02% myristic acid (C14:0) to 78.7% linoleic acid (C18:2). Gas chromatography (GC) analysis of the methyl esters of fatty acids in *Morus nigra* seeds revealed linoleic acid (C18:2), followed by palmitic acid (C16:0), oleic acid (C18:1), and stearic acid (C18:0), which together constituted approximately 97% of the total fatty acids identified. According to this study, the high C18:2 content (average 73.7%) indicates that black mulberry seed oil is a significant source of the essential fatty acid linoleic acid. Conversely, *Morus nigra* seeds contained relatively lower amounts of linolenic acid (C18:3) (0.3-0.5%), and 0.17 to 0.20 mg of α-tocopherol per 100 grams of *Morus nigra* seed oil. Major sterols in *Morus nigra* seed oil included β-sitosterol, D5-avenasterol, D5,23-stigmastadienol, clerosterol, sitosterol, and D5,24-stigmastadienol. The study concluded that black mulberry seed oil could be a highly valuable dietary substance.

Yılmaz and Durmaz (2015) examined the oil contents of different mulberry seed varieties, analyzing radical scavenging capacity, tocopherol, and total phenolic content. They observed that the oil content of mulberry seeds varied between 1645 and 2587 mg/kg, with differing δ-tocopherol levels. The second most common tocopherol homolog was γ-tocopherol, found in concentrations ranging from 299 to 854 mg/kg. The oil was found to contain 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, with α-tocopherol equivalent (α-TE) ranging from 1013 to 1743 mg/kg, and very high antioxidant capacity in freeze-dried samples with 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (FD-ABTS) radical cation tests showing 2574 to 4522 mg α-TE/kg. The study concluded that mulberry seed oil has higher antioxidant capacity and total phenolic content compared to mulberry pomace oil, with linoleic acid being the predominant fatty acid (66-80%).

Overall, defatted mulberry seeds contain approximately 30% protein, with 28.69% of this being essential amino acids. Although there are some challenges in the development and use of plant proteins, defatted mulberry seed protein is considered to have significant untapped potential compared to other fruits (Yao et al., 2020). Therefore, defatted mulberry seed proteins are suggested as a viable raw material source for producing edible antioxidant peptides. Expanding the application areas of mulberry seed protein and increasing protein extraction efficiency are crucial. Traditional extraction methods have disadvantages such as low yield, time-consuming processes, low energy efficiency, high cost, and low peptide yield. In contrast, ultrasonic-

assisted extraction is a low-cost, high-efficiency, and environmentally friendly method often used to extract active components like essential oils, proteins, and bioactive compounds from animal and plant sources (Sicaire et al., 2016; Sanwal et al., 2022; Pan et al., 2022).
Additionally, a study conducted in Bangladesh analyzed the physicochemical properties, glycerides, lipids, and fatty acid composition of mulberry seed oil. It was observed that the oil from mulberry seeds is orange-yellow and contains 31.41% oil. The triglyceride content ranged from 90.08% to 91.05%, while diglycerides ranged from 3.25% to 4.10%, and monoglycerides from 1.47% to 2.05%. Saturated and unsaturated fatty acids in mulberry seed oil varied between 15.04% and 16.28%, and 74.29% and 75.86%, respectively. The oil was found to contain a high percentage of linoleic acid (74.29%), followed by palmitic acid (10.60%), stearic acid (5.61%), and a very small amount of myristic acid (0.07%) (Rahman et al., 2014).

USING OF MULBERRY SEEDS IN THE FOOD INDUSTRY

The oil extracted from mulberry seeds contains a high proportion of unsaturated fatty acids, making it suitable for use in food products. Particularly, its high oleic acid content provides health benefits and allows for its use in various food products, including as a cooking oil or in salad dressings and margarine. As a protein source, mulberry seeds are valuable due to their high protein content (approximately 25-40%). This protein content can be utilized in the food industry for high-protein dietary supplements, sports nutrition products, and various processed foods. Additionally, peptides derived from mulberry seeds can be used in functional food products. These peptides may exhibit antioxidant, antimicrobial, and other biological activities, making them suitable for use in health-beneficial functional food products. Furthermore, they can contribute to various food products through their flavouring and aroma-enhancing properties. Moreover, components derived from mulberry seeds can be used as food additives. For instance, mulberry seed extracts rich in polyphenols can be employed to extend the shelf life of food products and prevent oxidative deterioration. Mulberry seeds, with their high fibre content, can be used as a fibre supplement in food products. Fibre supports digestive health and can be added to various processed foods. The utilization of waste generated during the processing of mulberry seeds can enhance environmental sustainability. These wastes can be used as animal feed or for environmental applications such as composting. Mulberry seeds also have potential applications in various biotechnological processes. For example, some active components can be involved in the production of probiotic products and other food products that enhance biological activities. The potential of mulberry seeds in the food industry is significant not only in terms of nutritional value but also for its health benefits and environmental sustainability. Therefore, research and development efforts focused on mulberry seeds could offer innovative and valuable applications across various sectors of the food industry (Orhan et al., 2020; Merz et al., 2020; Gómez-Mejía et al., 2021; Sanwal et al., 2022).
Fresh foods, particularly meat products, produce ammonia and volatile gases during storage, causing pH changes (Zhang et al., 2019). This has led to a growing need for natural pH-sensitive pigments derived from plants in packaging materials due to the potential health hazards associated with synthetic chemical dyes (Wang et al., 2019). In this context, various sources of anthocyanins, such as black rice bran (Ge et al., 2020), carrots (Goodarzi et al., 2020), jambolan (*Syzygium cumini*) fruits (Merz et al., 2020), purple sweet potatoes (Chen et al., 2020), and hibiscus (roselle) (Zhang et al., 2019), are being explored for pH indicator-based materials for packaging to monitor and preserve food quality.
Mulberry pomace, which constitutes approximately 40% of the total fruit weight as a byproduct of mulberry juice processing, is a rich source of anthocyanins (Garg et al., 2019; Liu et al., 2019). Evaluating mulberry pomace extract as a film-forming component helps reduce food waste and contributes to the development of advanced functional natural food packaging

materials. Zhang et al. (2021) conducted a study using free and microencapsulated mulberry pomace extracts combined with psyllium seed gum to develop active and pH-sensitive food packaging films. They proposed that natural pH indicator-based smart packaging films, derived from enriched psyllium seed gum and microencapsulated mulberry pomace extracts, could be healthier and more promising alternatives compared to synthetic films.

Lee et al. (2011) examined the antioxidant activities and contents of polyphenolic compounds isolated from the methanolic extracts of defatted mulberry seeds from three mulberry varieties ('Daesungppong', 'Iksuppong', and 'Cheongilppong') using a range of column chromatographic methods, including silica gel, Sephadex LH-20, and ODS-A. They found that eleven polyphenolic compounds exhibited antioxidant activities, with rutin (IC50 = 20.2 μM), isorhamnetin (IC50 = 22.5 μM), quercetin (IC50 = 24.6 μM), quercetin (IC50 = 27.8 μM), (+)-dihydroquercetin (IC50 = 28.9 μM), and chlorogenic acid (IC50 = 30.6 μM) showing stronger antioxidant activities compared to L-ascorbic acid (IC50 = 31.5 μM) and α-tocopherol (IC50 = 52.3 μM). However, protocatechuic acid (IC50 = 68.2 μM) showed lower activity, while (+)-dihydrokaempferol (IC50 = 33.8 μM), trans-resveratrol (IC50 = 36.2 μM), morin (IC50 = 47.6 μM), and 4-prenyl morin (IC50 = 48.2 μM) exhibited moderate antioxidant activities. The levels of these eleven polyphenolic compounds in mulberry seeds varied among the three varieties: rutin (31.1–60.0 mg/100 g) > quercetin (7.2–34.2 mg/100 g) > (+)-dihydroquercetin (13.2–33.1 mg/100 g) > quercetin (15.8–19.5 mg/100 g) > 4-prenylmorin (10.5–43.3 mg/100 g) > isorhamnetin (5.8–15.4 mg/100 g) > chlorogenic acid (0.0–15.3 mg/100 g) > morin (4.7–7.2 mg/100 g) > procatechuic acid (0.0–11.6 mg/100 g) > (+)-dihydrokaempferol and trans-resveratrol (<0.1 mg/100 g). The 'Daesungppong' variety showed higher flavonoid content, particularly rutin and quercetin derivatives, while the 'Iksuppong' variety had the highest levels of phenolic acids and morin derivatives. The 'Cheongilppong' variety contained fewer polyphenolic compounds compared to the other two varieties. These results suggest that mulberry seeds with antioxidant polyphenolic compounds could be used as potential anti-diabetic, anti-hypertensive, and anti-aging agents in functional foods and cosmetics.

Furthermore, due to its high oleic acid content, mulberry seed oil is suggested for use in the industry for products such as soap, cosmetics, body massage oil, and dye preparation, as well as for artificial feed production for poultry, fish, and cattle (Gecgel et al., 2011). Additionally, the utilization of seed waste from wine and mulberry processing offers a simple, natural, and cost-effective alternative for obtaining bioactive compounds, which can be converted into high-value products for food, pharmaceutical, cosmetic, and other industries. The total market value of products derived from fruit and grape food waste in Europe and North America is expected to exceed 4 billion dollars by 2020 (Ghate et al., 2019).

Moreover, the health benefits of mulberry and grape seed wastes, particularly their antioxidant-rich components, and their potential to support a circular economy through waste valorization are crucial aspects of sustainable strategies to reduce waste in the current food industry. The potential use of mulberry extracts, for instance, as preservatives to extend the shelf life of food products or as health agents in the pharmaceutical sector, underscores the importance of effectively and sustainably utilizing these by-products. This approach aids in making mulberry juice and wine production more cost-effective and sustainable while generating strategies for the recovery of valuable compounds from these residues (Gómez-Mejía et al., 2021).

China, one of the leading producers of mulberries, has a total annual mulberry production of approximately 6.5 million tons, with this amount expected to increase further. Additionally, in China, mulberry seeds are used in large quantities at low cost as by-products in the juice and wine industries. Mulberry seeds have high nutritional value (Oh et al., 2013). The oil content of mulberry seeds is 30-40%, which is comparable to that of high-oil crops like sesame. Furthermore, mulberry seed oil is dominated by unsaturated fatty acids (UFAs), constituting 87.5% of total fatty acids, with linoleic acid (LA) making up approximately 80% of this proportion, which is higher than that of general edible oils. However, the content of alpha-linolenic acid (ALA) in mulberry seed oil is relatively low (McCusker and Grant-Kels, 2010). The need to explore effective energy-saving technologies due to resource and energy scarcity highlights the urgency of finding new ways to utilize mulberry seeds efficiently (Lonnie et al., 2020).

SIGNIFICANT OF MULBERRY SEEDS FOR HUMAN HEALTH

Studies indicate that mulberry seeds contain high levels of antioxidant components, particularly polyphenols and flavonoids. These compounds are known to combat free radicals, reduce oxidative stress, and slow down cellular ageing. Antioxidants play a significant role in reducing the risk of chronic diseases such as cardiovascular diseases and cancer. In this context, mulberry seeds contain healthy fatty acids, especially oleic acid, which supports cardiovascular health. Unsaturated fatty acids help regulate cholesterol levels, lower blood pressure and reduce the risk of heart diseases. Components in mulberry seeds also exhibit anti-inflammatory properties. Chronic inflammation is associated with numerous health issues, and thus mulberry seeds are reported to improve overall health by reducing inflammation. The high fibre content of mulberry seeds supports the digestive system, with fibre regulating bowel movements, preventing constipation, and enhancing digestive health. Mulberry seeds, as a low glycemic index food, may assist in regulating blood sugar levels. Additionally, some studies have indicated that mulberry seed extracts show protective effects against diabetes. Mulberry seeds contain vitamins (e.g., vitamin E) and minerals (e.g., zinc) that support skin health. These nutrients can improve skin elasticity, reduce signs of ageing, and address skin issues. Vitamins and minerals in mulberry seeds contribute to a strengthened immune system, with components such as vitamin C and zinc enhancing immune responses and protecting the body against infections. Due to its high fibre content, mulberry seeds may increase satiety and aid in weight management. Fibre slows digestion and helps maintain a feeling of fullness for longer periods. Mulberry seeds also contain omega-3 fatty acids and antioxidants that support brain health, potentially improving brain function and reducing the risk of neurological diseases. Due to these health benefits, mulberry seeds are a valuable addition to the diet but should be consumed as part of a balanced diet with a variety of foods to achieve the best nutritional outcomes (Kadam et al. 2019; Yao et al. 2020; Gómez-Mejía et al. 2021; Rahman and Islam 2021; Maqsood et al. 2022).

Mulberry seed oil is obtained by pressing the seeds. Research indicates that over 70% of the oil content in mulberry seeds is composed of linoleic acid. Since linoleic acid is highly beneficial for human health and can be used as a potential functional lipid source, its preservation is crucial. However, due to the high levels of polyunsaturated fatty acids in mulberry seed oil, it is prone to oxidation. To prevent this, researchers have explored the use of acidolysis as an alternative method for effective emulsification and antioxidant purposes. This method involves using moderately asidolized egg albumin, which has been reported to enhance protein activity. However, enzymatic hydrolysis, although a method for increasing target protein activity, is limited by the cost, activity, and stability of the enzymes used (Chang et al. 2018).

Lectins play a role as recognition molecules in many biological processes, including the regulation of glycoprotein intracellular trafficking, the attachment of infectious agents to host cells, the recruitment of leukocytes to inflamed areas, metastasis, and cell interactions in the immune system. Understanding how lectins bind to carbohydrates at the molecular level could facilitate the design and production of drugs for combating infections, inflammation, and cancer (Harmankaya et al. 2014). In this context, lectins purified from mulberry seeds, despite similarities to sugars in content, differ significantly in terms of molecular mass, subunit structure, neutral sugar content, and sugar composition compared to most lectins purified from other sources. Mulberry seed lectins have been found to exhibit toxic properties similar to those of lectins extracted from *Abrus precatorius*, *Ricinus communis*, and *mistletoe* (Yeasmin et al. 2001). Consequently, research is ongoing into their potential use in the development of carbohydrate-binding and anti-cancer drugs.

Lectins isolated from mulberry seeds using ConA Sepharose affinity chromatography were purified to obtain a mannose/glucose-specific lectin. This lectin was found to be monomeric, with a molecular weight of 22,000 as determined by SDS-PAGE. The lectin obtained from mulberry seeds is a glycoprotein with a neutral sugar content of 28.57%, identified as mannose and glucose. The lectin agglutinated rat red blood cells, and D-mannose and D-glucose were found to be inhibitors in hapten inhibition tests. Additionally, the lectin exhibited cytotoxic effects in the saltwater shrimp lethality bioassay, with N-terminal sequences identified up to residue 45, excluding positions 21, 39, 42, and 44. It is noted that the production of monomeric mannose-specific lectins from mulberry seeds is significant due to the carbohydrate (38%), fat (32%), and protein (15%) content, highlighting their importance as a source for lectin production (Absar et al. 2005).

Influenza viruses have become a significant infectious disease in recent years, causing seasonal outbreaks and pandemics, which pose severe threats to human health and the global economy. Alongside vaccines, various strategies are employed to combat this pathogen, with recent studies highlighting plant-derived anti-influenza components. Among these components are polyphenols, such as quercetin-3-gallate, cardiotonic glycosides from *Adenium obesum*, teaflavin derivatives, catechins, resveratrol, chlorogenic acid, and dendrobin (Thapa et al. 2012; Li et al. 2017). *Morus alba*, belonging to the *Moraceae* family, contains 1-deoxynojirimycin, which has antiviral effects against hepatitis B and C viruses (Jacob, et al. 2007). Research has indicated that *Morus alba* fruit juice and seeds possess antiviral properties against foodborne viral analogues (Oh et al. 2013; Lee et al. 2014). However, the antiviral activities of *Morus alba* fruit juice and seeds against influenza viruses have not been previously investigated. Studies have explored the antiviral effects of *Morus alba* fruit juice and seeds against influenza virus strains A/Brisbane/59/2007(H1N1) (BR59), pandemic A/Korea/01/2009(H1N1) (KR01), A/Brisbane/10/2007(H3N2) (BR10), and B/Florida/4/2006 (FL04). *Morus alba* fruit juice showed dose-dependent significant antiviral effects against BR59, KR01, and FL04 strains and exhibited noteworthy antioxidant activity (Kim and Chung, 2018). *Morus alba* juice demonstrated 0.2 log inhibitory effects against BR59 and KR01 at a 1% concentration, and 0.8 log inhibitory effects against FL04, a type B virus, at the same concentration. At 2% and 4% concentrations, it showed over 1.3 log inhibition against FL04, while displaying 0.5 and 0.4 log inhibition against BR59 and KR01, respectively, at 4% concentration. For *Morus alba* seeds, 0-0.4 log inhibition was observed at 50 μg/mL and 0.2-0.6 log inhibition at 100 μg/mL against BR59, KR01, BR10, and FL04. Particularly, *Morus alba* juice exhibited strong inhibitory effects against FL04 with 1.1 and 1.3 log reductions at 2% and 4% concentrations, respectively, whereas *Morus alba* seeds showed no antiviral activity against influenza viruses under similar treatment conditions (Kim and Chung, 2018).

Numerous studies have investigated the potential applications of mulberry seed extract in both food and health contexts. Noroviruses are among the most common causes of foodborne viral gastroenteritis, responsible for approximately 90% of non-bacterial outbreaks globally. The antiviral effects of mulberry seed extract (MAS) against foodborne viral analogues, namely feline calicivirus-F9 (FCV-F9) and murine norovirus-1 (MNV-1), were assessed using plaque assays and reverse transcription polymerase chain reaction (RT-PCR). Polyphenol compound analysis conducted via liquid chromatography-mass spectrometry identified caffeic acid, 3,4-dihydroxybenzoic acid, rutin, and cyanidin-3-rutinoside as the primary components of MAS, in decreasing order of abundance. The fraction with a molecular weight below 1 kDa (MAS-F1), except for caffeic acid, was found to have a similar but minor composition. When FCV-

F9 and MNV-1 were added to cells and simultaneously incubated with MAS or MAS-F1, the maximum antiviral effect was achieved in studies conducted on rats. MAS-F1 also showed a dose-dependent significant reduction in the expression of MNV-1 or FCV-F9 polymerase genes, with cyanidin-3-rutinoside found to be effective in reducing MNV-1 polymerase gene expression among the polyphenols. MAS or MAS-F1 inhibited viral infection on foodborne viral analogues, demonstrating effectiveness at the initial stage of viral replication. Therefore, mulberry seed extract has been reported as a potential candidate for antiviral food substances and for its anti-noroviral activity (Oh et al., 2013).

Grape and mulberry extracts are known to have high content of organic acids, particularly oxalic and quinic acids, whereas mulberry seeds are richer in tocopherols, primarily γ-tocopherol. Phenolic compounds are predominant in grape seed residues, mostly existing as catechin trimers and dimers. These phenolic compounds have also been detected in lower concentrations in mulberry seeds, indicating that both types of waste are readily available sources of catechins and their conjugates. Additionally, mulberry seeds contain significant amounts of ellagic acid derivatives, which are likely to play a crucial role in inhibiting lipid peroxides and hydroperoxides and in antimicrobial activity. Regarding bioactivities, antioxidant activity tests have shown that mulberry seed extract is more effective in inhibiting the formation of thiobarbituric acid reactive substances (TBARS) and erythrocyte membrane hemolysis. Studies have also identified similar antimicrobial activity in grape and mulberry extracts, with effectiveness against methicillin-resistant *Staphylococcus aureus* (MRSA). Furthermore, no cytotoxic effects were observed against tumour and normal cells at a concentration of 400 µg/mL, demonstrating that these extracts are not effective for cytotoxic purposes (Gómez-Mejía et al., 2021). Among the primary bioactive compounds of mulberry are alkaloids, flavonoids, polyphenols, and polysaccharides (Hao et al., 2022). Among polyhydroxylated alkaloids, 18 alkaloid derivatives isolated from mulberries have been identified, with over 72% of the total alkaloid content being 1-deoxynojirimycin (DNJ) (Asano et al., 2001). 1-Deoxynojirimycin (DNJ) is a polyhydroxy piperidine alkaloid with a D-glucose-like structure that competitively inhibits α-glucosidase activity (Butt et al., 2008) and exhibits anti-diabetic (Kimura et al., 2007), anti-viral (Warfield et al., 2017), and anti-tumour (Lou et al., 2010) effects. In nature, DNJ is predominantly found in the mulberry plant and, though at very low concentrations, also in *Streptomyces* and some *Bacillus* species. The yield of DNJ isolated from dried mulberry material has been reported as 1.650 mg/g from roots, 0.840 mg/g from fruits, 0.690 mg/g from leaves (Asano et al., 2001), and 6.015 mg/g from seeds (Yin et al., 2018). However, the DNJ content in mulberries varies not only between different tissues but also among different varieties (Lou et al., 2010).

Mulberry (*Morus alba* L.) plants are rich in 1-deoxynojirimycin (DNJ), a potential α-glucosidase inhibitor with various physiological activities (Hao et al., 2022). Compared to other tissues, *Morus alba* L. seeds have the highest content of 1-deoxynojirimycin; however, the biosynthesis mechanisms of DNJ remain unclear. Xin et al. (2024), conducted a study on DNJ biosynthesis in seeds from 27 mulberry varieties and found that variety MS02 had the highest DNJ level (22.28 mg/g), while variety MS15 had the lowest DNJ level (0.37 mg/g). Their study identified four key enzymes involved in DNJ biosynthesis: polyphenol oxidase, tyrosine aminotransferase, aromatic-L-amino-acid decarboxylase, and tropinone reductase. They concluded that DNJ biosynthesis in mulberry seeds occurs through the upregulation of these enzymes. The findings from these studies are crucial for further research on the biosynthesis of this important medicinal compound.

Mulberry seeds contain significant amounts of protein (25% to 40% fat) and essential amino acids (28.69% of total amino acids). Enhancing the bioavailability of these proteins could reveal the economic value of mulberry seeds. Nutritionists are concerned about the quality and functionality of protein-rich plants and are striving to extract proteins from these plants to increase their potential applications (Kumar et al., 2021; Lonnie et al., 2020). Bian et al. (2022), conducted a study on the production of biologically active sweet-flavoured peptides from mulberry seed protein using multifrequency countercurrent ultrasonic technology. They demonstrated that the protein yield from mulberry seeds is high and that the multifrequency ultrasonic method is an effective way to prepare sweet-flavoured peptides from mulberry seed meal. The study also suggested that these peptides could be good candidates for use as natural flavour enhancers in functional food products due to their activity and their inhibitory effects on the growth of HepG2 cells in vitro. However, the relationship between the biological activities and flavour characteristics of these peptides is not yet fully understood, indicating that further research on mulberry seed peptides is necessary.

CONCLUSION AND RECOMMENDATIONS

Mulberry seeds, which are rich in protein (25-40%) and contain high levels of essential amino acids, have significant potential for use in the food industry. The oil derived from mulberry seeds is also notable for its high content of unsaturated fatty acids (UFAs), particularly oleic acid, making it beneficial for health and suitable for use in cooking oils, salad dressings, and margarine. Additionally, mulberry seeds are a valuable source of peptides with antioxidant, antimicrobial, and other biological activities, which can be utilized in functional food products and as natural flavor enhancers. The seeds are also high in polyphenols, which can extend the shelf life of food products and prevent oxidative spoilage. Furthermore, mulberry seeds contain dietary fibre that supports digestive health and can be used as a supplement in processed foods. The processing of mulberry seed waste for use in animal feed or compost contributes to environmental sustainability. Overall, mulberry seeds have significant potential in various biotechnological processes, including the production of probiotic products and other biologically active food items, highlighting their importance not only for their nutritional value but also for their health benefits and environmental impact.

In this study, it is recommended that a more comprehensive analysis of the phytochemical components of mulberry seed oil be conducted. Specifically, comparing the phytochemical profiles of oils obtained from various *Morus* spp. could aid in identifying components that might optimize specific health benefits. Further detailed research is needed to explore the potential applications of mulberry seed oil in the food industry. Notably, studies should continue on its use as a food additive, its potential as a natural preservative, and its impact on flavour and texture improvement in different food products. Clinical trials are required to evaluate the effects of mulberry seed oil on human health. Research involving human subjects is crucial for validating the antioxidant, anti-inflammatory, and other health benefits of this oil. Additionally, it is important to investigate how to utilize the waste generated during the production of mulberry seed oil and how these wastes can contribute to environmental sustainability. Studies could focus on the use of waste materials in composting or as animal feed. The development of new technologies and methods for extracting and processing mulberry seed oil is recommended. Specifically, the advancement of cost-effective and energy-efficient methods could enhance the widespread applicability of this oil. Educating consumers about the health benefits and potential applications of mulberry seed oil in the food industry could facilitate broader adoption. These recommendations could provide a significant foundation for better understanding and optimizing the potential of mulberry seed oil in both the health and food industries.

REFERENCES

Abbas, G. M., F. M. A. Bar, H. N. Baraka, A. A. Gohar and M. F. Lahloub, (2014). A New Antioxidant Stilbene and Other Constituents from The Stem Bark of *Morus nigra* L. Natural Product Research 28(13):952–959.

Abbasi, A. M., M. H. Shah, G. XinBo and N. Khan, (2016). Comparison of Nutritional Value, Antioxidant Potential, and Risk Assessment of The Mulberry (*Morus*) Fruits. International Journal of Fruit Science 16(2):113–134.

Absar, N., Yeasmin, T., Raza, M. S., Sarkar, S. K., and Arisaka, F. (2005). Single step purification, characterization and N-terminal sequences of a mannose specific lectin from mulberry seeds. The Protein Journal, 24, 369-377.

Argon, Z. Ü., İlhan, N., Gökyer, A., Öztürk, S. B., ve Koparal, B. (2019). Phytochemical evaluation of *Morus alba* seeds and cold pressed oil. Journal of the Turkish Chemical Society Section A: Chemistry, 6(1), 41-50.

Asano, N., Yamashita, T., Yasuda, K., Ikeda, K., Kizu, H., Kameda, Y., and Ryu, S.K. (2001). Polyhydroxylated alkaloids isolated from mulberry trees (*Morus alba* L.) and silkworms (*Bombyx mori* L.). Journal of Agricultural and Food Chemistry, 49, 4208–4213.

Barnes, R. L. (2008). *Morus* L. The woody plant seed manual. Agriculture Handbook, 727, 728-732.

Biasiolo, M., Da Canal, M. T., ve Tornadore, N. (2004). Micromorphological characterization of ten mulberry cultivars (*Morus* spp.). Economic Botany, 58(4), 639-646.

Bian, Y. R., Li, W. J., Pan, L. H., Peng, Q. M., You, S., Sheng, S., and Wu, F. A. (2022). Sweet-flavored peptides with biological activities from mulberry seed protein treated by multifrequency countercurrent ultrasonic technology. Food Chemistry, 367, 130647.

Butt MS, Nazir A, Sultan MT, Schroën K. (2008). *Morus alba* L. nature’s functional tonic. Trends Food Sci Technol. 19:505–512.

Chang, J., Kang, X., end Yuan, J. (2018). Enhancing emulsification and antioxidant ability of egg albumin by moderately acid hydrolysis: Modulating an emulsion-based system for mulberry seed oil. Food Research International, 109, 334–342.

Chen, S., Wu, M., Lu, P., Gao, L., Yan, S., and Wang, S. (2020). Development of pH indicator and antimicrobial cellulose nanofibre packaging film based on purple sweet potato anthocyanin and oregano essential oil. International Journal of Biological Macromolecules, 149, 271-280.

Chrysochou, P., Tiganis, A., Trigui, I. T., and Grunert, K. G. (2022). A cross-cultural study on consumer preferences for olive oil. Food Quality and Preference, 97, 104460.

Das, B.K., Mukherjee, S.K. (1986) Promotion of femaleness in *Morus alba* (L) by multiple application of plant growth regulators. Geobios 13:272–273.

Harmankaya, A., Özcan, A., ve Harmankaya, S. (2014). Lektinler ve glikobilimlerdeki önemi. Kafkas Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 7(1), 1-14.

Hao, J., Gao, Y., Xue, J., Yang, Y., Yin, J., Wu, T., and Zhang, M. (2022). Phytochemicals, pharmacological effects and molecular mechanisms of mulberry. Foods, 11(8), 1170.

Garg, N., Yadav, P., and Kumar, S. (2019). Simultaneous extraction of pectin, fibre, anthocyanin and oil from mulberry pomace.

Ge, Y., Li, Y., Bai, Y., Yuan, C., Wu, C., and Hu, Y. (2020). Intelligent gelatin/oxidized chitin nanocrystals nanocomposite films containing black rice bran anthocyanins for fish freshness monitorings. International Journal of Biological Macromolecules, 155, 1296-1306.

Gecgel, U., Velioglu, S. D., and Velioglu, H. M. (2011). Investigating some physicochemical properties and fatty acid composition of native black mulberry (*Morus nigra* L.) seed oil. Journal of the American Oil Chemists Society, 88, 1179–1187.

Ghate, V. S., Zhou, W., and Yuk, H. G. (2019). Perspectives and trends in the application of photodynamic inactivation for microbiological food safety. Comprehensive Reviews in Food Science and Food Safety, 18(2), 402-424.

Goodarzi, M. M., Moradi, M., Tajik, H., Forough, M., Ezati, P., and Kuswandi, B. (2020). Development of an easy-to-use colorimetric pH label with starch and carrot anthocyanins for milk shelf-life assessment. International Journal of Biological Macromolecules, 153, 240-247.

Gómez-Mejía, E., Roriz, C. L., Heleno, S. A., Calhelha, R., Dias, M. I., Pinela, J., and Barros, L. (2021). Valorisation of black mulberry and grape seeds: Chemical characterization and bioactive potential. Food Chemistry, 337, 127998.

Jacob, J. R., Mansfield, K., You, J. E., Tennant, B. C., and Kim, Y. H. (2007). Natural iminosugar derivatives of 1-deoxynojirimycin inhibit glycosylation of hepatitis viral envelope proteins. Journal of Microbiology, 45(5), 431-440.

Kadam, R. A., Dhumal, N. D., and Khyade, V. B. (2019). The Mulberry, *Morus alba* (L.): The medicinal herbal source for human health. Int. J. Curr. Microbiol. Appl. Sci, 8(4), 2941-2964.

Kimura T, Nakagawa K, Kubota H, Kojima Y, Goto Y, Yamagishi K, Oita S, Oikawa S, Miyazawa T. (2007). Food-grade mulberry powder enriched with 1-deoxynojirimycin surpresses the elevation of postprandial blood glucose in humans. J Agric Food Chem. 55(14):5869–5874.

Kim, E. O., Yu, M. H., Lee, Y. J., Leem, H. H., Kim, S., Kang, D. H., and Choi, S. W. (2010). Comparison of functional constituents and biological activity of the seed extracts from two mulberry fruits. Preventive Nutrition and Food Science, 15(2), 98-104.

Kim, H., and Chung, M. S. (2018). Antiviral activities of mulberry (*Morus alba*) juice and seed against influenza viruses. Evidence-Based Complementary and Alternative Medicine, 2018(1), 2606583.

Lee, Y. J., Kim, E. O., and Choi, S. W. (2011). Isolation and identification of antioxidant polyphenolic compounds in mulberry (*Morus alba* L.) seeds. Journal of the Korean Society of Food Science and Nutrition, 40(4), 517-524.

Lee, J. H., Bae, S. Y., Oh, M., Kim, K. H., and Chung, M. S. (2014). Antiviral effects of mulberry *(Morus alba*) juice and its fractions on foodborne viral surrogates. Foodborne pathogens and disease, 11(3), 224-229.

Liang, L., Wu, X., Zhu, M., Zhao, W., Li, F., Zou, Y., and Yang, L. (2012). Chemical composition, nutritional value, and antioxidant activities of eight mulberry cultivars from China. Pharmacognosy magazine, 8(31), 215.

Li, R., Liu, T., Liu, M., Chen, F., Liu, S., and Yang, J. (2017). Anti-influenza A virus activity of dendrobine and its mechanism of action. Journal of agricultural and food chemistry, 65(18), 3665-3674.

Li, W., Liu, X., Wang, J., Wu, J., Sheng, S., Wu, F., et al. (2020). Synthesis and characterization of structural lipids with a balanced ratio of n-6/n-3 from mulberry seed oil and α-linolenic acid using a microfluidic enzyme reactor. Food and Bioproducts Processing, 120, 21–32

Liu, Y., Qin, Y., Bai, R., Zhang, X., Yuan, L., and Liu, J. (2019). Preparation of pH-sensitive and antioxidant packaging films based on κ-carrageenan and mulberry polyphenolic extract. International journal of biological macromolecules, 134, 993-1001.

Lonnie, M., Laurie, I., Myers, M., Horgan, G., Russell, W. R., and Johnstone, A. M. (2020). Exploring health-promoting attributes of plant proteins as a functional ingredient for the food sector: a systematic review of human interventional studies. Nutrients, 12(8), 2291.

Lou DS, Fan T, Wang CY, Yan H, Gui ZZ. (2010). Isolation of 1-deoxynojirimycin from silkworm powder and its inhibitory effect on tumour cells. Acta Sericol Sinica. 36:109–114.

Kumar, M., Tomar, M., Potkule, J., Verma, R., Punia, S., Mahapatra, A., … Kennedy, J. F. (2021). Advances in the plant protein extraction: Mechanism and recommendations. Food Hydrocolloids, 115, 106595.

Mahesh, D. S., Vidhathri, B. S., Vidyashree, D. N., Narayanaswamy, T. K., Subbarayappa, C. T., and Muthuraju, R. (2017). Biochemical composition and pharmacological properties of mulberry (*Morus* spp.)-a review. International Journal of Current Microbiology and Applied Sciences, 6(7), 2207-2217.

Maqsood, M., Anam Saeed, R., Sahar, A., and Khan, M. I. (2022). Mulberry plant as a source of functional food with therapeutic and nutritional applications: A review. Journal of food biochemistry, 46(11), e14263.

Merz, B., Capello, C., Leandro, G. C., Moritz, D. E., Monteiro, A. R., and Valencia, G. A. (2020). A novel colorimetric indicator film based on chitosan, polyvinyl alcohol and anthocyanins from jambolan (*Syzygium cumini*) fruit for monitoring shrimp freshness. International Journal of Biological Macromolecules, 153, 625-632.

McCusker, M. M., and Grant-Kels, J. M. (2010). Healing fats of the skin: the structural and immunologic roles of the ω-6 and ω-3 fatty acids. Clinics in dermatology, 28(4), 440-451.

Oh, M., Bae, S. Y., and Chung, M. S. (2013). Mulberry (*Morus alba*) seed extract and its polyphenol compounds for control of foodborne viral surrogates. Journal of the Korean Society for Applied Biological Chemistry, 56, 655-660.

Orhan, E., Meleksen, A. K. I. N., Eyduran, S. P., and Ercisli, S. (2020). Molecular characterization of mulberry genotypes and species in Turkey. Notulae botanicae horti agrobotanici Cluj-Napoca, 48(2), 549-557.

Özgen, M., Serçe, S., ve Kaya, C. (2009). Phytochemical and antioxidant properties of nthocyanin-rich *Morus nigra* and *Morus rubra* fruits. Scientia horticulturae, 119(3), 275-279.

Pan, L. H., Peng, Q. M., Li, W. J., Yan, C. H., Li, Z. N., You, S., and Wang, J. (2022). Antioxidant peptides derived from mulberry seed protein by ionic liquid-enhanced microfluidic hydrolysis with immobilized protease. Biomass Conversion and Biorefinery, 12(10), 4435-4447.

Rahman, M. M., Akther, A., Moinuddin, M., Yeasmin, M. S., Rahman, M. M., Rahman, M. S., ve Sayeed, M. A. (2014). Investigation some physicochemical properties, lipids, glycerides and fatty acid composition of mulberry (*Morus alba* L.) seed oil of three different regions of Bangladesh. Am. J. Appl. Chem, 2, 38-41.

Rahman, M. S., and Islam, S. M. S. (2021). Studies on Food, Health and Environmental Perspectives In Mulberry (*Morus* spp.)—A Review. J. Bio-Sci, 29, 163-179.

Sanwal, N., Mishra, S., Sahu, J. K., and Naik, S. N. (2022). Effect of ultrasound-assisted extraction on efficiency, antioxidant activity, and physicochemical properties of sea buckthorn (*Hippophae salicipholia*) seed oil. Lwt, 153, 112386.

Sbihi, H. M., Nehdi, I. A., Mokbli, S., Romdhani-Younes, M., and Al-Resayes, S. I. (2018). Hexane and ethanol extracted seed oils and leaf essential compositions from two castor plant *(Ricinus communis* L.) varieties. Industrial Crops and Products, 122, 174-181.

Sicaire, A. G., Vian, M. A., Fine, F., Carré, P., Tostain, S., and Chemat, F. (2016). Ultrasound induced green solvent extraction of oil from oleaginous seeds. Ultrasonics Sonochemistry, 31, 319-329.

Thapa, M., Kim, Y., Desper, J., Chang, K. O., and Hua, D. H. (2012). Synthesis and antiviral activity of substituted quercetins. Bioorganic and medicinal chemistry letters, 22(1), 353-356.

Vijayan, K., Saratchandra, B., and da Silva, J. A. T. (2011). Germplasm conservation in mulberry (*Morus* spp.). Scientia Horticulturae, 128(4), 371-379.

Wang, X., Yong, H., Gao, L., Li, L., Jin, M., and Liu, J. (2019). Preparation and characterization of antioxidant and pH-sensitive films based on chitosan and black soybean seed coat extract. Food hydrocolloids, 89, 56-66.

Warfield KL, Warren TK, Qiu XG, Wells J, Mire CE, Geisbert JB, Stuthman KS, Garza NL, Van Tongeren SA, Shurtleff AC, et al. (2017). Assessment of the potential for host-targeted iminosugars UV-4 and UV-5 activity against filovirus infections in vitro and in vivo. Antiviral Res. 138:22–31.

Xin, X., Jiang, X., Thomas, A., Niu, B., Zhang, M., Xu, X., and Gui, Z. (2024). Studies on 1-deoxynojirimycin biosynthesis in mulberry (*Morus alba* L.) seeds through comparative transcriptomics. Natural Product Research, 38(15), 2585-2594.

Yao, X. H., Shen, Y. S., Hu, R. Z., Xu, M., Huang, J. X., He, C. X., and Chen, T. (2020). The antioxidant activity and composition of the seed oil of mulberry cultivars. Food Bioscience, 37, 100709.

Yeasmin, T., Tang, M. A. K., Razzaque, A., and Absar, N. (2001). Purification and characterization of three galactose specific lectins from mulberry seeds (*Morus* sp.). European Journal of Biochemistry, 268(23), 6005-6010.

Yilmaz, M. A., and Durmaz, G. (2015). Mulberry seed oil: A rich source of δ-tocopherol paper. Journal of the American Oil Chemists Society, 92, 553–559.

Yin H, Ye JJ, Wang XJ, Wu JX, Xia CL, Zhang HR, Feng J, Cui WZ. (2018). Content determination of active ingredients in mulberry seed and hypoglycemic effect on diabetic model mice. Sci Sericul. 44(5):0738–0745.

Chang, J., Kang, X., and Yuan, J. L. (2018). Enhancing emulsification and antioxidant ability of egg albumin by moderately acid hydrolysis: Modulating an emulsion-based system for mulberry seed oil. Food Research International, 109, 334-342.

Zhang, J., Zou, X., Zhai, X., Huang, X., Jiang, C., and Holmes, M. (2019). Preparation of an intelligent pH film based on biodegradable polymers and roselle anthocyanins for monitoring pork freshness. Food chemistry, 272, 306-312.

Zhang, X., Zhao, Y., Shi, Q., Zhang, Y., Liu, J., Wu, X., and Fang, Z. (2021). Development and characterization of active and pH-sensitive films based on psyllium seed gum incorporated with free and microencapsulated mulberry pomace extracts. Food Chemistry, 352, 129333.

THE REMOVAL OF TOXIC METALS FROM CONTAMINATED ENVIRONMENTS BY BIOSORPTION

Numan YILDIRIM[1], Gokhan Onder ERGUVEN[2]*

[1]*Munzur Unıversıty, Tuncelı Vocatıonal School, Department of Plant And Anımal Productıon, Organıc Agrıculture Pr., Tunceli, Turkey*
[2] *Munzur University, Faculty of Economics and Administrative Sciences Department of Political Science and Public Administration Department of Urbanization and Environmental Issues, Tunceli, Turkey*

**Corresponding author e-mail: gokhanondererguven@gmail.com*

ABSTRACT

Environmental pollution is a significant global issue, especially in areas exposed to metal pollution. Remediation techniques for such areas often incur high costs. An alternative and highly effective method involves using biological molecules rather than physical and chemical methods to remove metals from industrial waste. Applications of biological molecules for metal removal include biosorption, adsorption, and phytoremediation methods. Biosorption is the process of metal ion uptake from aqueous environments by biomass. The dissolved substances on the surface of the biosorbent biomass must pass through the film of its surrounding liquid solvent. Optimal conditions are required for the biosorption process, including factors such as the type of metal ion, the amount and type of biomass, concentration, temperature, and pH of the solution, all of which influence the biosorption method.

Keywords: Remediation, Biological molecules, Biosorption, Biomass

INTRODUCTION

As technological advancements continue to evolve, so do the requirements for raw materials. Strategic raw materials (SRMs), such as those found in smartphones, electric vehicles, magnets, and low-carbon technologies like wind turbines and solar panels, play a crucial role in modern technologies and economic development (Ferro and Bonollo, 2019). The shift towards green technologies, crucial for attaining net-zero emissions, heavily relies on strategically important raw materials (SRMs) integrated into these technologies. Nations prioritizing this transition are embracing circular economy strategies to manage energy consumption and enhance recycling rates of SRMs (Alliance, 2021). In Turkey, the proliferation of policy frameworks for renewable and low-carbon technologies indicates a steep rise in demand and criticality of SRMs (Balcilar et al., 2023). The heightened demand for these materials raises issues such as supply risk and economic significance (Martins and Castro, 2019).

The term "criticality" refers to the combination of high economic importance and a high risk of supply disruption (Buijs et al., 2012). Assessing the raw materials on which countries heavily depend, particularly those with limited reserves, is essential for development.

The demand for strategic raw materials (SRMs) continues to rise with the increasing activities in advanced technology sectors. However, there are concerns about potential supply interruptions for certain raw materials like silver, antimony, and gold in the coming years (Zanoletti et al., 2021).

SRMs hold a pivotal position across diverse industries and national economies owing to their economic significance, indispensable applications, innovative capabilities, and vulnerability in terms of supply. They are integral components in sectors ranging from electronics and renewable energy systems to defense equipment, material recycling, CO2 capture and utilization, sustainable biomaterials, and agricultural production.

Metals such as lithium, cobalt, manganese, nickel, aluminum, and copper have gained considerable attention for their essential roles in enabling cleaner energy technologies and sustainable practices (Han et al., 2023). Biosorption, an often overlooked method in wastewater treatment, offers a cost-effective approach for removing strategic raw materials (SRMs) from wastewater. This method requires less processing cost, energy, and chemicals, and it also boasts shorter processing times compared to traditional techniques (Olawale, 2019). Biosorption involves the use of living or non-living biomass to effectively remove contaminants from wastewater.

Indeed, biosorption is a rapid and reversible process in which ions from aqueous solutions bind to various functional groups present on the surface of biomass. This mechanism allows for efficient removal of contaminants from water, making biosorption an effective technique in wastewater treatment and environmental remediation processes. Unlike some other methods, biosorption doesn't depend on cellular metabolism and can be effective for removing various types of metals (Davis et al., 2003). Environmental sustainability, cost-effectiveness, and abundance of biomass have indeed made biosorption an attractive option for various applications. This method offers a promising approach for removing contaminants from aqueous solutions while minimizing costs and environmental impact, making it a valuable tool in wastewater treatment and pollution remediation efforts (Lee and Park, 2012).

According to Ibrahim et al., (2012), Various biomass sources, including byproducts from industries like rice, wheat, corn, and wood, as well as living organisms such as microorganisms have been considered suitable biosorbent materials (Ibrahim et al., 2018). Biosorption processes typically involve ion exchange, microprecipitation events, complexation and adsorption, are fast and reversible.

Overall, biosorption is recognized as a cost-effective biotechnological method for treating low-concentration, high-volume wastewater containing metals, utilizing biomaterials like bacteria, crab shells, fungi, and algae. It involves the accumulation of biological and waste materials in aqueous solutions on or inside the cell surface.

Biosorption of strategical raw materials

One of the physicochemical processes in which specific biological substances passively bind pollutants to their cellular structures is Biosorption. The biomaterial that operates through this mechanism is actually a biological environment that enables the binding of ions from aqueous solutions to functional structures on the biomass surface. The rapid development of this process is characterized by its reversibility. Microorganisms used for biosorption are mostly plant-derived materials and are produced from waste products, biopolymers and environmentally friendly materials. It consists of materials obtained from various easily available sources.

Multiple interactions that enable ions to bind to functional groups on the surface of the biosorbent occur through biosorption. This means the accumulation of strategic raw materials. Since this method is carried out with simple processes without the need for additional nutrients and energy, its cost is also low. According to the definition in Razzak et al. (2022), bioadsorption is another term used for the removal of undesirable elements with the help of microorganisms and is a very effective method for removing SRMs from the waste environment with minimal chemical or biological wastewater treatment.

The mechanism of SRM pollutant removal through biosorption occurs in a step-by-step manner, and it offers an affordable option that can be completed in a very short time. Additionally, bioadsorbents are recyclable, and there are various types available that can be tailored for specific metals of interest.

Factors Affecting Biosorption

The kinetics of biosorption in SRM recovery are influenced by various factors. Optimal conditions must be established for the biosorption process to occur effectively. Initially, dissolved substances targeted for retention on the biosorbent surface must pass through the surrounding solvent liquid film, a process known as film diffusion. Following this, the substances must undergo pore diffusion to enter the inner parts of the pores in the biosorbent material. The final stage involves the binding of the dissolved substance to the biosorbent material.

The amount of element bound to biomass in biosorption methods is affected not only by the type of biomass but also by physicochemical factors such as concentration, temperature, amount of biomass, and solution pH (Volesky, 2004). These factors play crucial roles in determining the efficiency and effectiveness of the biosorption process for SRM recovery.

Effect of pH

According to Deniz and Karabulut (2017), pH is an important parameter in affecting the biosorption of SRMs as it affects the surface charge of the solvent and also affects its mobility. High hydrogen ion concentration in the solvent inhibits the binding activity of metal cations to the adsorbent material at low pH levels. Lee and Park (2012) also explain the effect of pH during biosorption in more detail. According to them, lower pH levels cause functional groups in the biomass to be protonated with more hydrogen ions, and as a result, the negative charge density decreases and the electrostatic attraction of metal ions decreases. Considering the opposite of this situation, increasing pH values causes deprotonation of hydrogen ions attached to functional groups, and as a result of this situation, biosorption of SRM cations becomes easi

Another factor that affects the biosorbent material and the load of adsorbate molecules is the pH of the wastewater. Fluctuations in pH affect the speciation and solubility of some pollutants as well as electrostatic forces. Optimizing pH settings means increasing the adsorption properties of certain adsorbate types. In addition, another impressive critical factor is the contact time between the biosorbent and the wastewater. Sufficient contact time paves the way for mass transfer and spreading of adsorbate molecules onto the biosorbent surface. One of the critical conditions of this situation is that excessive contact time may not always lead to high adsorption, which may negatively affect the overall efficiency of the purification process (Sarma et al., 2019).

Effect of Temperature

The effect of temperature on adsorption is inevitable. It is known that high temperatures generally lead to increased adsorption rates. Therefore, temperature also affects the concentration of adsorbate molecules and the binding ability between the adsorbate and the biosorbent. The negative effect of this situation may vary depending on the specific adsorbate and biosorbent system. Additionally, degradation and competition in wastewater is an important variable that may compete for adsorption sites rather than the intended adsorbate. Interference from competing ions, dissolved organic matter, or other contaminants also inhibits adsorption ability or selectivity. As a result of this situation, understanding the wastewater content and potential degradation is also very important for designing optimum biosorbents.

The amount or dosage of biosorbent used in the current treatment system also has a significant impact on the adsorption efficiency. While insufficient amounts of biosorbent lead to the removal of the pollutant at low efficiencies, high doses may be uneconomic (Chen et al., 2020). As a result of this situation, some experimental studies or process modeling should be preferred in order to determine the optimum amount of biosorbent for effective treatment efficiency.

Turbulence and flow velocity

Turbulence and flow rate are important in continuous flow systems where agitation can affect contact time and mixing of biosorbents. Optimum flow rates and agitation are required to ensure the interaction between adsorbent and biosorbent while minimizing the risk of inadequate mass transfer. It is necessary to carefully examine and optimize these factors according to the characteristics of the target pollutants in the wastewater that need to be eliminated and the selection of biosorbent materials. A detailed characterization study and column studies provide significant insight into adsorption behavior and lead to the development of efficient wastewater treatment technologies.

Effect of biomass dosage

According to their research in Sarada et al., (2017), the amount of biomass is essential in determining the presence of surface area of biosorbents and binding sites for SRM ions. Increasing the biomass dosage typically allows the affinity between the algal cell wall and SRM ions to strengthen (Ibrahim et al., 2018). However, there may be a saturation point where the removal percentage stabilizes or decreases due to the aggregation of biosorption sites, leading to a reduction in the available surface area for SRM ions to bind (Kayalvizhi et al., 2022).

Accoding to Ibrahim et al. (2018), contact time is indeed a critical factor in determining biosorption capacity Many experiments have been carried out to establish the optimal contact time necessary for biomass to reach equilibrium with SRM solutions.

Effect of SRM concentration on biosorption.

The initial concentration of SRM ions can indeed have a significant impact on the removal percentage. Moreover, temperature plays a crucial role in the biosorption process. Temperature increases can promote chemical absorption. In this case, physical intervention cannot be neglected. Fluctuations due to increases in temperatures are associated with thermodynamic processes occurring between metals and biomass. This may bring to mind that biosorption is an endothermic reaction (Lucaci et al., 2020). We can summarize the temperature dependence of adsorption as increasing temperature, which can activate more regions on the biomass surface and expand the pores on the biosorbent material, leading to a higher reaction rate. According to Kayalvizhi et al. (2022), this underlines the importance of temperature in increasing the efficiency of biomass as a biosorbent (Ciobanu et al., 2023).

Adsorption isotherms

Biomass analysis is crucial for understanding its properties related to biosorption and its affinity to metals, including surface characteristics. Adsorption isotherm studies provide valuable insights into the biosorption behavior of biomass. Various studies have utilized Langmuir, Freundlich, and Temkin isotherm models to analyze the adsorption efficiency of metals such as As, Zn, Cr, and Pb by biomass, yielding information about adsorbed metals and aqueous concentration at equilibrium (qe and Ce, respectively) (DeMessie et al., 2022).

The Langmuir isotherm model elucidates sorption characteristics on homogeneous solid surfaces through monolayer adsorption, describing the adsorption rate and sites occupied by the adsorbate. It effectively explains the homogeneity of binding sites and the uniformity of energy used for sorption on active sites (Alavi et al., 2018). AlHazmi et al. (2022) further detailed the Langmuir model and concluded that the adsorption balance on the adsorbent surface is dynamic and the adsorbed substances do not come into direct contact.

Proctor and Toro-Vazquez, (1996) conducted that, the Freundlich isotherm, characterized by nonlinear sorption, elucidates the heterogeneous surface characteristics of the adsorbent biomass, explaining the exponential distribution between energy used for sorption and sorption sites. This reversible equilibrium adsorption occurs rapidly at a fixed temperature conditions.

The temkin isotherm model examines the interaction between the adsorbates and adsorbents to be adsorbed and is also related to the free energy of sorption according to the surface coverage. It indicates how the heat of adsorption of whole adsorbates or related molecules decreases linearly as the coverage area of the adsorbent surface increases. According to Piccin et al. (2011), the adsorption process is expressed as a consistent distribution of binding energies until the maximum binding energy is reached. This developed and mentioned model also follows an effective process in determining the linear decrease in the adsorption heat and the uniform distribution of the binding energy up to the maximum energy level (Inyinbor et al., 2016).

Adsorption kinetics

The data regarding biosorption kinetics offer valuable insights into the rate at which SRMs are adsorbed onto the surface of biomass, providing crucial information for optimizing the biosorption process. Understanding these kinetics allows for the identification of optimal conditions for efficient SRM removal. The movement of solute particles from the concentrated solution to the surface of the adsorbent through various mechanisms such as intra-particle transport and adsorption on the inner surface of the pores according to the liquid film diffusion law represents the biosorption process. (Shakir et al., 2010). In this mechanism of action, the adsorbent is formed rapidly and significantly in the interior of the biomass, in the capillary spaces and pores. As a result, this contributes to the overall efficiency of the process.

The kinetics of biosorption is affected by the mass transport rate of the particles as well as the chemical and physical properties of the biomass used for adsorption. These factors play a crucial role in determining the effectiveness of SRM removal. Additionally, biomass undergoes significant changes in its surface morphology and characteristics after biosorption, further highlighting the dynamic nature of the process (Shakir et al., 2010). Overall, biosorption kinetics provide essential insights into the mechanisms and efficiency of SRM removal, guiding the development of effective wastewater treatment strategies.

RESULTS AND DISCUSSION

Recent studies have highlighted the significant biosorption capacity of lichens. For instance, Uluozlu et al. (2008) investigated the biosorption of Pb(II) and Cr(III) from aqueous solutions using the lichen species Parmelina tiliaceae. Ekmekyapar et al. (2006) studied the biosorption of copper(II) from the non-living biomass of Cladonia rangiformis. Sari et al. (2007) examined the biosorption of Pb(II) and Ni(II) from aqueous solutions using the lichen species Cladonia furcata. Bingöl et al. (2009) explored the biosorption of chromate ions from aqueous solutions using the lichen species Cladonia rangiformis. Yalcin et al. (2010) investigated the biosorption of Cu^{2+} and Zn^{2+} by the lichen species Rocella phycopsis.

Uluözlü et al. (2010) focused on the biosorption of antimony (III) using the lichen species Physcia tribacia.

Biosorption, also known as the removal of metal ions using dead biomass in a solution, occurs because the surfaces of organisms are negatively charged, enabling them to adsorb positively charged metal ions (Volesky, 2004). Microorganisms can take up metals both actively (through acclimated cells) and passively (via biosorption). Research indicates that passive uptake is more prevalent in biosorption methods compared to active uptake, as living systems (active uptake) require frequent addition of additional nutrients, thereby increasing the chemical oxygen demand or biological oxygen demand or at the reaction outlet (Hussein et al., 2004).

CONCLUSION

Absolutely, biosorption indeed stands out as a promising technology for environmental remediation. Its remarkable adsorption capacity for various pollutants, coupled with its versatility in targeting a broad spectrum of contaminants, makes it a valuable tool in the ongoing effort to address environmental pollution. Harnessing the power of biological substances such as algae, fungi, or bacteria, biosorption offers an effective means of removing pollutants from water or air. Moreover, its cost-effectiveness, especially in scenarios involving low to moderate levels of contamination, further enhances its appeal as an environmentally friendly solution. Overall, biosorption holds significant potential in safeguarding ecosystems and human health while contributing to sustainable environmental management.

REFERENCES

Alavi S.A., Zilouei H., Zargoosh K., Asadinezhad A., Yousefi Abdolmaleki A. 2018. Surface modification of *Nizimuddinia zanardini* and *Stoechospermum marginatum* using 4-phenyl-3 thiosemicarbazide to improve heavy metals biosorption from water. IJEST, 15: 993-1000.

AlHazmi G.A., AbouMelha K.S., El-Desouky M.G., El-Bindary A.A. 2022. Effective adsorption of doxorubicin hydrochloride on zirconium metal-organic framework: equilibrium, kinetic and thermodynamic studies. J. Mol. Struct. 1258, Article 132679.

Alliance G. 2021. Critical Point Securing the Raw Materials Needed for the UK's Green Green Alliance. United Kingdom.

Ayub A., Raza Z.A., Majeed M.I., Tariq M.R., Irfan A. 2020. Development of sustainable magnetic chitosan biosorbent beads for kinetic remediation of arsenic contaminated water. Int. J. Biol. Macromol. 163: 603-617.

Ayub A., Raza Z.A., Majeed M.I., Tariq M.R., Irfan A. 2020. Development of sustainable magnetic chitosan biosorbent beads for kinetic remediation of arsenic contaminated water. Int. J. Biol. Macromol. 163: 603-617.

Balcilar M., Uzuner G., Nwani C., Bekun F.V. 2023. Boosting energy efficiency in Turkey: the role of public–private partnership investment. Sustain. Times, 15(3): 2273.

Bingol A, Aslan A., Cakici A. 2009. Biosorption of chromate anions from aqueous solution by a cationic surfactant-modified lichen (*Cladonia rangiformis* (L.)). J. Hazard Mat. 161: 747-52.

Buijs B., Sievers H., Tercero Espinoza L.A. 2012. Limits to the critical raw materials approach. Proceedings of the Institution of Civil Engineers-Waste and Resource Management, ICE Publishing, 201-208.

Chen X., Zhao Y., Zhang C., Zhang D., Yao C., Meng Q., Zhao R., Wei Z. (2020). Speciation, toxicity mechanism and remediation ways of heavy metals during composting: a novel theoretical microbial remediation method is proposed. J. Environ. Manag., 272, Article 111109.

Ciobanu A.A., Bulgariu D., Ionescu I.A., Puiu D.M., Vasile G.G., Bulgariu L. 2023. Evaluation of thermodynamic parameters for Cu (II) ions biosorption on algae biomass and derived biochars Symmetry. 15: 1500.

Davis T.A., Volesky B., Mucci A. 2003. A review of the biochemistry of heavy metal biosorption by brown algae. Water Res. 37: 4311-4330.

DeMessie J.A., Sorial G.A., Sahle-Demessie E. 2022. Removing chromium (VI) from contaminated water using a nano-chitosan–coated diatomaceous earth. Sep. Sci. Technol., 15: 163-176.

Deniz F., Karabulut A. 2017. Biosorption of heavy metal ions by chemically modified biomass of coastal seaweed community: studies on phycoremediation system modeling and design Ecol. Eng. 106: 101-108.

Ekmekyapar F, Arslan A, Bayhan YK, Cakici A. 2006. Biosorption of copper (II) by nonliving lichen biomass of Cladonia rangiformis Hoffm J Hazard Mat. 137: 293-8.

Ferro P., Bonollo F. 2019. Materials selection in a critical raw materials perspective. Mater. Des., 177, Article 107848.

Han S., Zhenghao M., Meilin L., Xiaohui Y., Xiaoxue W. 2023. Global supply sustainability assessment of critical metals for clean energy technology. Resour. Policy, 85, Article 103994.

Hussein H., Ibrahim S.F., Kandeel K., Moawad H. 2004. Biosorption of heavy metals from wastewater using Pseudomonas sp. Electron. J. Biotechn. 7: 38-46.

Ibrahim W.M., Aziz Y.S.A., Hamdy S.M., Gad N.S. 2018. Comparative study for biosorption of heavy metals from synthetic wastewater by different types of marine algae. J. Bioremediat. Biodegrad. 9(): 425.

Ibrahim W.M., Aziz Y.S.A., Hamdy S.M., Gad N.S. 2018. Comparative study for biosorption of heavy metals from synthetic wastewater by different types of marine algae. J. Bioremediat. Biodegrad. 9(1): 425.

Inyinbor A.A., Adekola F.A., Olatunji G.A. 2016. Kinetics, isotherms and thermodynamic modeling of liquid phase adsorption of Rhodamine B dye onto Raphia hookerie fruit epicarp. Water Resour. Ind. 15: 14-27.

Kayalvizhi K., Alhaji N.M.I., Saravanakkumar D., Mohamed S.B., Kaviyarasu K., Ayeshamariam A., Elshikh M.S. 2022. Adsorption of copper and nickel by using sawdust chitosan nanocomposite beads–a kinetic and thermodynamic study. Environ. Res. 203: Article 111814.

Lee S.H., Park C.H. 2012. Biosorption of heavy metal ions by brown seaweeds from southern coast of Korea. Biotechnol. Bioprocess Eng. 17: 853-861.

Lee S.H., Park C.H. 2012. Biosorption of heavy metal ions by brown seaweeds from southern coast of Korea. Biotechnol. Bioprocess Eng.,17: 853-861.

Lu T., Cao W., Liang H., Deng Y., Zhang Y., Zhu M., Ma W., Xiong R., Huang C. 2022. Blow-Spun nanofibrous membrane for simultaneous treatment of emulsified oil/water mixtures, dyes, and bacteria. Langmuir, 38: 15729-15739.

Lucaci A.R., Bulgariu D., Ahmad I., Bulgariu L. 2020. Equilibrium and kinetics studies of metal ions biosorption on alginate extracted from marine red algae biomass (*Callithamnion corymbosum* sp.). Polymers. 12: 1888.

Martins F., Castro H. 2019. Significance ranking method applied to some EU critical raw materials in a circular economy–priorities for achieving sustainability. Pro. Cir. 84: 1059-1062.

Olawale S.A. 2019. Biosorption of heavy metals: a mini review. ASAG, 3: 22-25.

Philip K., Jacob R., Gopalakrishnan J. 2021. Characterization of cassava root husk powder: equilibrium, kinetic and modeling studies as bioadsorbent for copper (ii) and lead (II). J. Encapsulation Adsorpt. Sci. 11: 69-86.

Piccin J.S., Dotto G.L., Pinto L.A.A. 2011. Adsorption isotherms and thermochemical data of FD&C Red n 40 binding by chitosan. Braz. J. Chem. Eng. 28: 295-304.

Proctor A.U.O.A., Toro-Vazquez J.F. (1996). The Freundlich isotherm in studying adsorption in oil processing. JAOCS, 73: 1627-1633.

Razzak S.A., Faruque M.O., Alsheikh Z., Alsheikhmohamad L., Alkuroud D., Alfayez A., Hossain S.M.Z., Hossain M.M. 2022. A comprehensive review on conventional and biological-driven heavy metals removal from industrial wastewater. Adv. environ. sci. 7, Article 100168

Sarada B., Krishna Prasad M., Kishore Kumar K., Murthy C.V. 2017. Biosorption of Cd^{+2} by green plant biomass, *Araucaria heterophylla*: characterization, kinetic, isotherm and thermodynamic studies. Appl. Water Sci. 7: 3483-3496.

Sarada B., Krishna Prasad M., Kishore Kumar K., Murthy C.V. 2017. Biosorption of Cd+ 2 by green plant biomass, Araucaria heterophylla: characterization, kinetic, isotherm and thermodynamic studies. Appl. Water Sci. 7: 3483-3496,

Sari A., Tuzen M., Uluozlı O.D., Soylak M. 2007. Biosorption of Pb(II) and Ni(II) from aqueous solution by lichen (Cladonia furcata) biomass. Biochem Eng J. 37: 151-8.

Sarma H., Sonowal S., Prasad M.N.V. (2019). Plant-microbiome assisted and biochar-amended remediation of heavy metals and polyaromatic compounds - a microcosmic study. Ecotoxicol. Environ. Saf. 176: 288-299.

Shakir K., Elkafrawy A.F., Ghoneimy H.F., Beheir S.G.E., Refaat M. (2010). Removal of rhodamine B (a basic dye) and thoron (an acidic dye) from dilute aqueous solutions and wastewater simulants by ion flotation. Water Res. 44: 1449-1461.

Uluözlü O.D., Sari A., Tuzen M., Soylak M. 2008. Biosorption of Pb (II) and Cr (III) from aqueous solution by lichen (Parmelina tiliaceae) biomass. Bioresource Technol. 99: 2972-80.

Volesky B. 2004. Sorption and Biosorption. St. Lambert, Quebec: BV Sorbex, Inc. 103-28.

Wentker M., Greenwood M., Asaba M.C., Leker J. 2019. A raw material criticality and environmental impact assessment of state-of-the-art and post-lithium-ion cathode technologies. J. Energy Storage, 26, Article 101022.

Yalcin E., Cavusoglu K., Kinalioglu K. 2010. Biosorption of Cu^{2+} and Zn^{2+} by raw and autoclaved Rocella phycopsis. J. Environ. Sci. 22(3): 367-73.

Zanoletti A., Cornelio A., Bontempi E. 2021. A post-pandemic sustainable scenario: what actions can be pursued to increase the raw materials availability? Environ. Res. 202, Article 111681.

AN OVERVIEW OF THE ACRIDIDAE FAMILY (ENSIFERA - ORTHOPTERA) IN THE VLORA REGION, SOUTH-WESTERN ALBANIA

Gerta Subashaj*[1]; Eltjon Halimi[2]; Dritan Topi[3], Anila Paparisto[4]

[1]*Reald University College, Faculty of Medical Technical Sciences, Vlora, Albania*

[2, 3,4]*University of Tirana, Faculty of Natural Sciences, Tirana, Albania*

Corresponding author e-mail: gerta.subashaj@fshnstudent.info

https://orcid.org/0009-0000-2112-4297

ABSTRACT

This study intends to provide a comprehensive account of the species of Short-Horned Grasshoppers belonging to the family Acrididae (Orthoptera) that may be discovered in the various habitats of the Vlora region, which is located in the southern part of Albania. A well-defined seasonal pattern that includes a cold and rainy winter and a warm and arid summer is characteristic of the climate in this region, which shows some characteristics that are typical of the Mediterranean climate. On a worldwide scale, this region represents a discovery that is both extraordinarily valuable and one of a kind. Following an examination of the data, it has been determined that the genera *Calliptamus*, *Chorthippus*, and *Oedipoda* are known to exist, with two species belonging to each of these genera. It is estimated that 13.33% of each species is present. For the purpose of establishing whether or not these species are present in this region, the Llogara and Armen National Parks were both investigated. According to the conclusions of the study, these parks, which each include six species and have a species frequency of forty percent, offer the best possible environment for the species in question.

Keywords: Orthoptera, Acrididae, Habitat, Biodiversity, Vlora, Southwestern Albania

INTRODUCTION

The Acrididae family, is a diversified group within the Orthoptera order. These grasshoppers are generally referred to as short-horned grasshoppers (Memon et al. 2021). According to (Riffat, 2015; Shaikh et al. 2018), the agricultural crops that they infest are considered to be pests if they occur. In addition to wetland environments, they may be discovered in a wide variety of habitats, including as grasslands, shrublands, herbaceous regions, and woods. (Dempster, 1963; Uvarov, 1966; Shaikh et al. 2018) all came to the conclusion that the majority of the species that belong to the Acrididae family are herbivorous insects.
The findings of the study conducted by (Akwanjoh, 2020) suggest that they have the potential to function as helpful bio-indicators for identifying changes in the environment. According to (Song et al. 2018), the family Acrididae is comprised of a wide variety of species that fall within the order Orthoptera and the suborder Caelifera.
Each and every member of the Acridiea family contains a pair of antennae that are extremely small and very thin. In contrast to the tympanum, which is situated on the lateral sides of the first abdominal segment, the tarsi are composed of three segments. The research conducted by

(Smith et al. 2014) revealed that certain adult species have wings, whereas others either do not have wings at all or have wings that are severely reduced. In this paper, we provide the results of our taxonomic investigation into the species belonging to the Acrididae family that were found in the Vlora region.

MATERIALS AND METHODS

During the years 2022 and 2023, the species were gathered from a variety of locations within the Vlora region, including National Park Llogara, Armen, and Selenica. The different species were gathered by the utilization of aerial entomological nets (NET). In order to maintain the color, the species were placed in tubes that contained ether (Colas, 2000; Halimi et al., 2023). Each tube was then labeled with the appropriate information, which included the date, location, coordinates, and altitude from the data collection. Notes were recorded in a journal regarding the vegetation, as well as any particular traits that were considered to be unique to particular species. For the taxonomic identification, the species were observed with a stereomicroscope (Perfex Sciences), and the keys from the publications for the countries bordering Albania, which offer similar climatic conditions and habitats to our study area, were used (Eades et al., 2014; Willemse et al. al., 2018; Willemes, 1985) as well as previous publications on the Order Orthopetra of Albania (Csiki, 1922; Ebner, 1910; Lemonnier – Darcemont & Darcemont, 2015; Subashaj, 2024).

RESULTS AND DISCUSSIONS

This study presents the species of the Acrididae family, which are listed in Table 1 according to the stations (Llogara, Armen, and Sevaster National Parks) in the habitats of the Vlora area, which is situated in the southwestern part of Albania, across the Ionian Sea, and in the southern Adriatic Sea. The Vlora area is located in the southern Adriatic Sea.

Table 1. List of species of the Acrididae family

No.	Specie	Llogara National Park	Armen	Sevaster
1	**Genus Chorthippus**			
1	*Chorthippus mollis mollis* Charpentier, 1825	+		
2	*Chorthippus bornhalmi* Harz, 1971	+		
2	**Genus Oedipoda**			
3	*Oedipoda caerulescens* Linnaeus, 1758	+	+	+
4	*Oedipoda germanica* Latreille, 1804	+		
3	**Genus Pezotetix**			
5	*Pezotettix giornae* Rossi, 1794	+		
4	**Genus Euchorthippus**			
6	*Euchorthippus declivus* Brisout, 1848	+		
5	**Genus Sphingonotus**			
7	*Sphingonotus caerulans* Linnaeus, 1767		+	
6	**Genus Pseudochorthippus**			
8	*Pseudochorthippus parallelus tenuis* Zetterstedt, 1821		+	
7	**Genus Paracaloptenus**			
9	*Paracaloptenus caloptenoides* Brunner von Wattenwyl, 1861		+	
8	**Genus Acrotylus**			
10	*Acrotylus patruelis* Herrich-Schaffer, 1838		+	
9	**Genus Acryptera**			
11	*Arcyptera sp* Serville, 1839		+	
10	**Genus Calliptamus**			
12	*Calliptamus sp* Serville, 1831			+
13	*Calliptamus italicus* Linnaeus, 1758			+
11	**Genus Oedaleus**			
14	*Oedaleus decorus* Germar, 1825			+
12	**Genus Acrida**			
15	*Acrida ungarica mediterranea* Herbest, 1786			+

Twelve genera and fifteen species are included in the collection of species belonging to the Acrididae family that was collected from three different sites (Table 2, Figure 1).

Table 2. Species and genus enlisted

Genus	Species number	Species frequency
Acrida	1	6.66%
Acrotylus	1	6.66%
Acryptera	1	6.66%
Calliptamus	2	13.33
Chorthippus	2	13.33
Euchorthippus	1	6.66%
Oedaleus	1	6.66%
Oedipoda	2	13.33
Paracaloptenus	1	6.66%
Pezotetix	1	6.66%
Pseudochorthippus	1	6.66%
Sphingonotus	1	6.66%

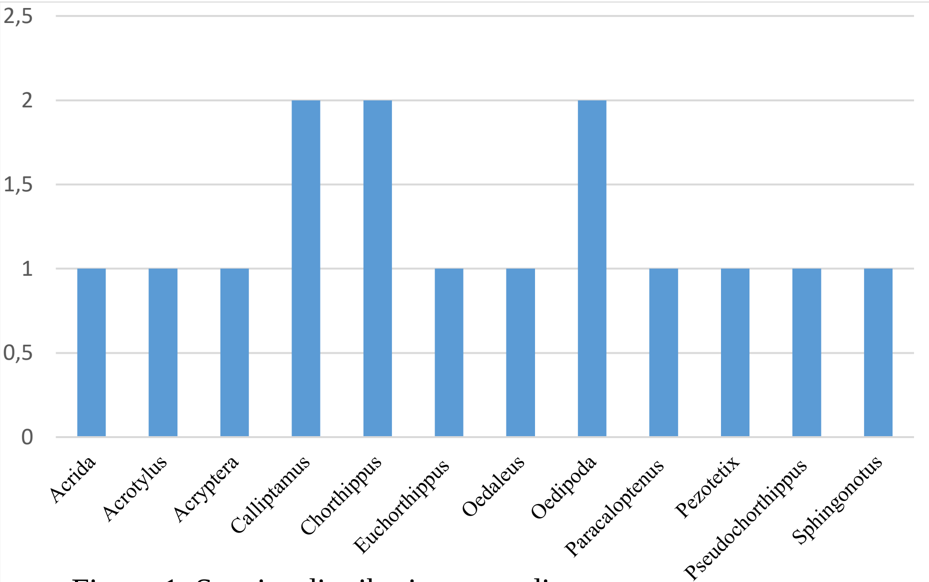


Figure 1. Species distribution according to genus

Based on the data, it can be deduced that the genera *Calliptamus*, *Chorthippus*, and *Oedipoda* are responsible for two species out of the total number of species. Thirteen point three percent of the total number of species in the entire collection is accounted for by these genera. The genera *Acrida, Acrotylus, Acryptera, Euchorthippus, Oedaleus, Paracaloptenus, Pezotetix, Pseudochorthippus*, and *Sphingonotus* are the ones that come in close succession after them. Due to the fact that each of these genera has one species, the total number of species that may be found in each of these genera is one. The frequency of each species within each genus is found to be six point six percent.
According to the findings of the inquiry into the diversity of stations, which are shown in table 3 and figure 2, the Llogara and Armen National Park stations each include six species, which accounts for forty percent of the total number of species. These findings are presented in the

table and figure respectively. Each of the five unique species that make the Sevasteri station their home has a frequency of 33.33 percent, and the station is home to all five of these species. The stations in Llogara and Armen National Park offer the most optimal environments for species that are members of the Acrididae family. As a consequence, the amount of disturbance that is generated by human activities is reduced to the greatest extent possible.

Table 3. Species according to the surveyed stations

Station	Species number	Species frequency
Parku Kombetar Llogara	6	40 %
Armen	6	40 %
Sevaster	5	33.33 %

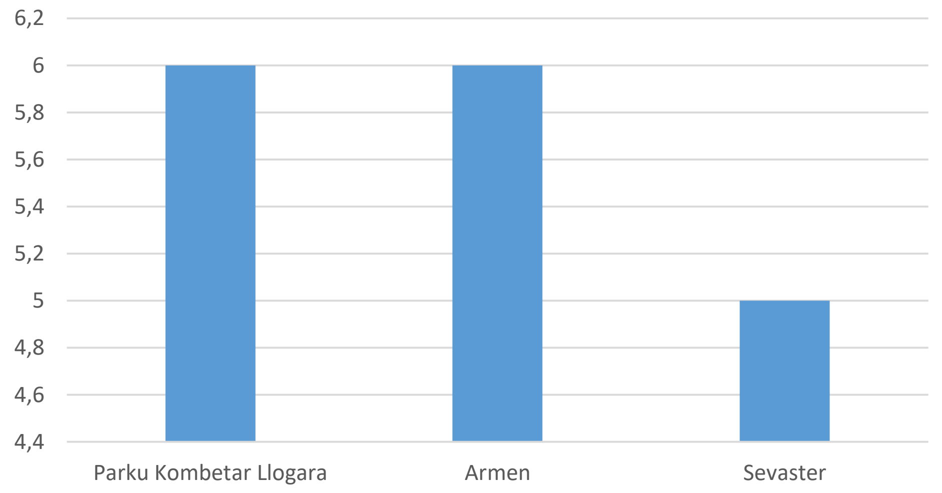


Figure 2. Species distribution according to studied

CONCLUSIONS

This research presents a complete analysis of the species that belong to the Acrididae family and are found in the ecosystems of the Vlora region. These species are found in the Vlora region. According to the parameters of this inquiry, we are discussing a total of twelve different genera and fifteen different species. A great degree of species diversity can be found within the Acrididae family, notably among the genera *Calliptamus*, *Chorthippus*, and *Oedipoda*, each of which has two different species. Acrididae is a family of insects. These three genera collectively are responsible for thirteen-point three percent of the total number of species that have ever been discovered. With a total of six species, the stations in Llogara and Armen National Park have the most species diversity of all of the stations in the park. This represents forty percent of the total species diversity count. According to the information shown here, it is possible to draw the conclusion that these stations provide the conditions that are most favorable for the species that belong to the Acrididae family.

ACKNOWLEDGMENTS

The authors wish to thank their French colleagues, M. Darcemont-Lemonnier and Ch. Darcemont, for their invaluable assistance in preparing this publication. Additionally, the University of Tirana provided support for this project through the "*UT-Search, Excellence, and Innovation—REI2023*" program.

REFERENCES

Akawanjoh, R., S., Tita, M., Nj. First Ever Data on the Occurrences of Acrididae (Acridoidea: Orthoptera) Grasshoppers in the North West Region of Cameroon. Journal of Biology and Life Science. 2020. 11/1.

Colas, G. Guide de l'entomologiste. Editions N. Boubée. 2000.

Csiki, E. Explorationes zoologicae ab C. Csiki in Albania peractae. VI. Orthopteren. Magyar Tudomanyos Akademia. 1922. 1, 75-82.

Dempster, JP. The population dynamic of Grasshoppers and Locust Reprinted from the Biological Review. 1963. 38:491-493.

Eades, D.C., Otte, M., Cigliano, M.M., Braun, H. Orthoptera Species File. 2014. Version 5.0/5.0.

Esler, K.J., A L. Jacobsen, and R. B. Pratt, 'Characteristics of Mediterranean-Type Ecosystems', The Biology of Mediterranean-Type Ecosystems, Biology of Habitats Series (Oxford, 2018; online edn, Oxford Academic), doi.10.1093/oso/9780198739135.003.0002.

Halimi, E., Paparisto, A., Alameti, E. Taxonomic and ecological data on the Hemiptera fauna from Berati region habitats in Albania. Journal of Biological. Research–Bollettino della Società Italiana di Biologia Sperimentale, 2023. 96(1).

Ebner, R. Ein Beitrag zur Orthopterenfauna der europäischen Türkei mit besonderer Berücksichtigung von Albanien (Ergebnisse einer Reise nach Nord-Albanien, A. Klaptocz, 1909). Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere. 1910. 29, 401-414.

Lemonnier – Daecemont, M., Darcemont, Ch. Peripodisma ceraunii (Orthoptera, Acrididae, Melanoplinae: Podismini), a new species of the genus Peripodisma from southern Albania. Articulata. 2015. 30, 53–62.

Memon, R., P., Panhwar, A. W. Study on some selected species of Acrididae (Orthoptera) from district Dadu, Sindh-Pakistan. Journal of Entomology and Zoology Studies. 2021. 9(1), 06-11.

Riffat, S. Wagan, M., S. Grasshoppers and locusts of Pakistan, Higher Education Commission, Pakistan. 2015. 1-180. ISBN: 978-969-417-180-7.

Smith, R., T., Froeba, G., J., Capinera, J. Key to the GRASSHOPPERS (Orthoptera: Acrididae) Of Florida. 2009. Florida Entomologist 87. 2004. 537-550. DOI:10.1653/00154040(2004)087[0537:KTTGOA]2.0.CO;2.

Song, H., Pérez, M., Woller., A., Cigliano., M. Evolution, Diversification, and Biogeography of Grasshoppers (Orthoptera: Acrididae). Insect Systematics and Diversity. 2018. 2(4): 3; 1–25.

Subashaj, G., Halimi, E., Paparisto, A. An Oreview of the Grasshoppers (Orthoptera, Insecta) in Vlore Area, Albania. International Journal of Advanced Multidisciplinary Research and Studies (Int. j. adv. multidisc. res. stud.). 2024. 4(3):316-319.

Shaikh, N., Sultana, R. Comparative study on the morphometric characteristics of egg-pods in various subfamilies of Acrididae. Journal of Entomology and Zoology Studies. 2018. 6(1), 1423-1426.

Uvarov, BP. Grasshoppers and Locust: A handbook of General Acridology, 1966.

Willemes, F. A key to the orthoptera species of Greece. Volume 2 Fauna Graeciae. Hellenic Zoological Society Athens. 1985. 288pp.

Willemse, L., Kleukers, R., Odé, B. The grasshoppers of Greece. EIS Kenniscentrum Insecten & Naturalis Biodiversity Center, Leiden. Naturalis Biodiversity Center. 2018. 440pp.

EVALUATION OF DEVELOPMENTAL TOXICITY OF LEAD (PB) ON ZEBRAFISH (*DANIO RERIO*) EMBRYOS

Duygu Özhan TURHAN
Department of Biology, Faculty of Arts and Science, Inonu University, Malatya 44280, Turkey

duygu.turhan@inonu.edu.tr

ABSTRACT

Heavy metals are of critical importance due to their easy absorption into the food chain and bioaccumulation processes. The aim of this study was to evaluate the toxic effects of lead (Pb) in zebrafish embryos. Embryos were exposed to 4.39-50 mg L^{-1} Pb for 96 hours and the survival rates, heart rates and embryonic growth rates of these individuals were determined. According to the results of the study, the 96-hour LC_{50} value of the Pb was determined as 17.99 (16.6-20.72) mg L^{-1}. Heart rates of zebrafish embryos (48-hour) exposed to the Pb were significantly reduced at concentrations of 14.81-50 mg L^{-1}. At concentrations of 6.88 mg L^{-1} and higher, it caused significant inhibition in the length of zebrafish larvae.

Keywords: Lead, zebrafish, embryo toxicity, pollution

INTRODUCTION

Today, one of the most important threats for all living things in the ecosystem is environmental pollution. (Sökmen et al., 2018).The direct introduction of pollutants into the water system also causes the destruction of the existing ecosystem. Heavy metals, as pollutants, create a toxic effect on the living system through the food chain when they enter the water ecosystem. Heavy metals are substances that are generally found in trace amounts in natural waters, but most of them are poisonous even at very low concentrations (Şavran and Küçük, 2022; Liu et al., 2017). The term heavy metal is a general term used for metals and metalloids that have a density higher than 5 g/cm^3 and show toxic effects even at low concentrations (Jarup, 2003). Heavy metals are included in the aquatic ecosystem, especially through natural resources and human activities (Sökmen et al., 2018).

Lead, one of the heavy metals, is commonly found in the forms of oxides, sulfides, acetates, chlorates and chlorides. Nowadays, lead contamination is increasing and its toxic effects are widely reported. The element lead binds strongly to some enzymes, amino acids, DNA and RNA, thus inhibiting metabolic pathways in the organism (Nassouhi, 2018). Lead has been reported to be a metal that has adverse effects on reproduction, development and behavior of fish (Ramsdorf et al., 2009)

D. rerio is one of the widely used model organisms in aquatic toxicology due to its low cost, small size and easy laboratory maintenance (Turhan, 2021).The aim of this study was to determine the different biological responses, such as lethality, growth and development, and heart rate, of zebrafish embryos after exposure to Lead (Pb) using the Fish Embryo Acute Toxicity Test (OECD, 2013).

MATERIAL AND METHOD

Testing organisms

Zebrafish embryos were used in this study. These embryos were obtained from adult fish reared in the Zebrafish Production System of the Aquatic Vertebrate Experimental Animals Unit of the Faculty of Arts and Sciences of Inonu University. In the zebrafish system with continuous water circulation, pH was 7.30, conductivity was 720 µS/cm, temperature was 28.2 °C and photoperiod was 14 h light and 10 h dark.

Zebrafish embryos were obtained with a filtered rearing system connected to the same aquatic characteristics as the main system and water circulation directly fed by the main system. Fertilized eggs were collected within 3 h and stored in an oven at 28.5 ºC in standard embryo water.

Chemicals and exposure

For fish embryo-toxicity testing, zebrafish embryos 6-8 hours after fertilization were exposed to Lead (Pb) at seven different concentrations (4.39-50 mg L^{-1}) for 96 hours in 96-well microplates. 250 µl of Pb solution prepared at different concentrations and a zebrafish embryo were added to each well. A total of 24 embryos were used for each concentration. Mortality rates of individuals examined with a stereomicroscope every 24 hours for a 96-hour period were recorded. At the 48th hour, the heart beats per minute of the embryos were determined.

At the end of the 96th hour, the malformation rates and malformation types of the surviving individuals were determined with a stereomicroscope, while their heights were measured using Euromex Image Focus 4.0 software.

Statistical Analysis

The statistical analysis of the collected data was conducted with SPSS and GraphPad Prism 5 (SPSS Inc., USA).

The results that did not comply with the required hypotheses for parametric tests were processed with the Mann-Whitney U and Kruskal Wallis test.

Probit regression analysis was used to determine the 96h mean lethal concentration (LC_{50}) for the embryos (EPA, ver. 1.5)

RESULT AND DISCUSSION

Heavy metals are very important for human and animal health. In recent years, heavy metals have been used very intensively and are passing into the aquatic environment (Gao et al. 2018). Therefore, in recent years, the use of biological data for heavy metals has been increasingly gaining interest. The main concern regarding the toxicology of heavy metals lies mainly in their chronic accumulation in aquatic environments. In this study, changes in embryo survival rate, and body length were analyzed to evaluate the possible toxicity on embryonic development of zebrafish exposed to lead (Pb) at concentrations between 4.39-50 mg L^{-1}.

96 hour LC_{50} values in *D. rerio* embryos exposed to Pb were calculated as 17.99 (15.65-20.72) mg L^{-1}. No mortality was observed at a concentration of 4.39 mg/L Pb. Mortality was

generally not observed over 24 hours in all exposures. Singh and Ansari (2017) rported that the LC_{50} 96-h value was determined as 21.63 in embryos exposed to lead.

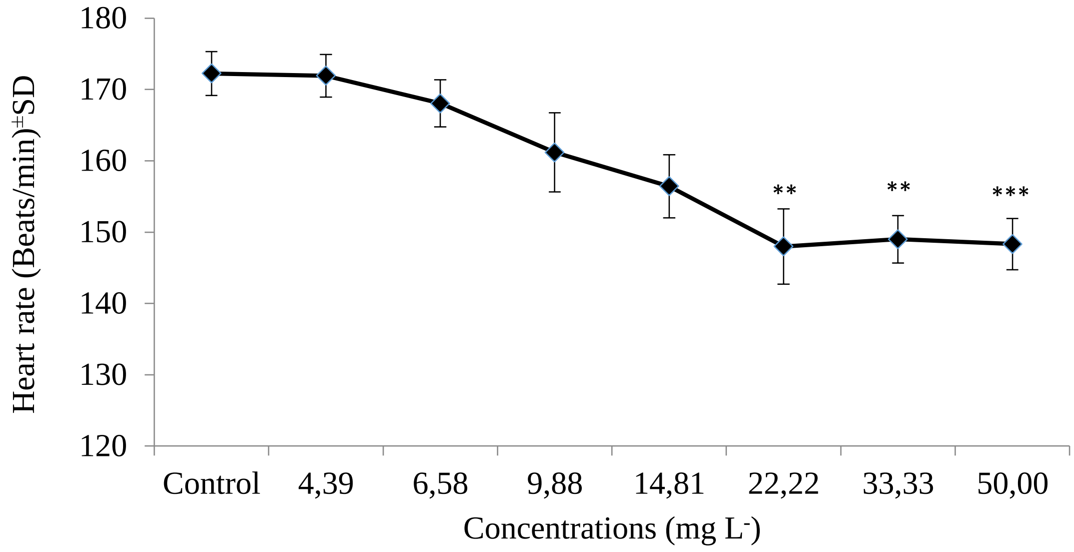


Figure 1. Heart rate in 96h *D. rerio* embryos exposed to Pb

** $p < 0.01$, *** $p < 0.001$

In this study, it was shown that zebrafish embryos exposed to Pb had a significant decrease in heart rate at 48 hours in a dose-dependent manner (Figure 1). There are many studies in the literature showing that pollution cause inhibition of heart rate in zebrafish embryos. Zhang et al. (2020) also reported that sublethal doses of pollutants can affect heart rate in zebrafish. At concentrations of 6.58 mg L^{-1} and higher, it caused significant inhibition in the length and heart rate of zebrafish larvae.

Table.1 Time-dependent mortality levels for *D. rerio* embryos exposed to different concentrations of Pb

		Σ (Mortalite)			
Consantrasyon (mg L^{-1})	n	24h	48h	72h	96h
Control	24	0	0	0	0
4.39	24	0	0	0	0
6.58	24	0	0	0	1
9.88	24	0	0	0	3
14.81	24	0	1	1	9
22.22	24	0	0	8	13
33.32	24	1	6	14	22
50.00	24	0	12	18	24

Especially at concentrations of 22.22-50 mg L^{-}heart beats per minute were significantly inhibited.

Determining body length change is an important parameter in evaluating the potential effects of pollutants on fish (Yang et al., 2018). Because the change in the length of fish significantly reflects many molecular and cellular responses that may occur in the individual depending on the effect of pollutants (Cook et al. 2005). In this study, a significant inhibition was observed in the body length of embryos exposed to Pb compared to the control group.

Table 2. Length of *D. rerio* larvae exposed to different concentrations of Pb for 96 hours

Concentrations	Pb			
	Length (mm)			
Control	3.75	±	0.06	
4.39	3.73	±	0.03	
6.58	3.71	±	0.04	
9.88	3.72	±	0.04	
14.81	3.68	±	0.05	
22.22	3.56	±	0.04	**
33.33	3.51	±	0.03	***

n: Living individual, ** $p< 0.01$, *** $p< 0.001$

Malformations were observed in all surviving individuals at concentrations of 22.22 and 33.32 mg $L^{-1.}$ Malformations such as pericardial edema (PE), yolk sac edema (YSE), tail deformation (TM) and spinal curvature (SC) were detected in zebrafish exposed to Pb. PE has been observed more frequently than other types of malformations. It has been reported that the occurrence of PE may be an indicator of abnormal cardiac development and deterioration of osmotic or metabolic functions.

CONCLUSIONS

This study showed that Pb caused dose-dependent developmental toxicity in zebrafish embryos. The effects that the potential accumulation of Pb in surface waters may cause on aquatic organisms were revealed from various aspects by this controlled experiment.

REFERENCES

Cook LW, Paradise CJ, Lom B 2005. The Pesticide Malathion Reduces Survival and Growth in Developing Zebrafish. Environmental Toxicology and Chemistry, 24(7): 1745-1750.

Jarup, L. (2003). Hazards of metal contamination. British Medical Bulletin, 68, 167-182.

OECD 236, Guidelines for the testing of Chemicals, Fish Embryo Acute Toxicity (FET) Test, 2013.

Gao Y., (2018) Concentration addition and independent action model: Which is better in predicting the toxicity for metal mixtures on zebrafish larvae. Science of the Total Environment 610–611 442–450

Liu T., Wang R., Cao H., Lin A., (2017). Polyaspartic acid alleviates heavy metal toxicity in zebrafish (*Danio rerio*). Chemistry and Ecology. 33:7 684-693.

Nassouhi, D. (2018). Kadmiyum, Kurşun Ve Kadmiyum-Kurşun Karışımına Maruz Bırakılan *Pistia stratiotes* L. Sucul Bitkisinin Fitoremediasyon Potansiyelinin Araştırılması Ankara Üniversitesi Fen Bilimleri Enstitüsü Yüksek Lisans Tezi.

Ramsdorf WA, Ferraro MVM, Oliveiraribeiro CA, Costa JRM, Cestari MM; Genotoxicity evaluation of different doses of inorganic lead (Pb II) in Hoplias malabaricus Environ. Monit. Assess., 2009; 158: 77-85

Singh, C.B., Ansari, B.a., (2017). Toxicity of two heavy metals Lead and Cobalt on Zebrafish, *Danio rerio*. Sch. Acad. J. Biosci., 5(9):682-687

Sökmen, T.Ö., Güneş , M, Kırıcı, M. (2018). Türk Tarım ve Doğa Bilimleri Dergisi 5(4): 578–588.

Şavran, G. ve Küçük, F. (2022). Sucul Canlılarda Ağır Metal Birikimi ve Etkileri, Akademia Doğa ve İnsan Bilimleri Dergisi, 8(1), 2022: 65-78

Turhan, D.Ö (2021). Evaluation of teratogenic and toxic effects of enrofloxacin-based antibiotic on zebrafish (*Danio rerio*) larvae with biochemical and developmental markers. *Chemistry and Ecology*, *37*(8), 704–714.

Yang X, Sun Z, Wang W, Zhou Q, Shi G, Wei F, Jiang G 2018. Developmental Toxicity of Synthetic Phenolic Antioxidants to The Early Lifestage of Zebrafish. Science of The Total Environment, 643, 559-568.

Zhang C, Zhang J, Zhu L, Du Z, Wang J, Wang J, Li B, Yang Y 2020. Fluoxastrobin-Induced Effects on Acute Toxicity, Development Toxicity, Oxidative Stress, and DNA Damage in *Danio rerio* Embryos. Science of the Total Environment, 715: 137069.

USE OF GINGER (*Zingiber officinale*) AND TURMERIC (C*urcuma lon*ga) IN BROILER DIETS

Taner İŞEVİ[1], Güray ERENER[1] Emrah GÜNGÖR[1], Şevket ÖZLÜ[1], Aydın ALTOP[1],

[1] Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Turkiye

Corresponding author e-mail: emrah.gungor@omu.edu.tr

ABSTRACT

The development of drug resistance has become a worldwide problem due to the hazardous use of antimicrobials in poultry feeding. Therefore, the use of biotic or natural products such as phytobiotics (phytogenics or botanicals) as a replacement for antibiotics has received great attention. In recent years, phytobiotics or their components have been recognized as a new class of natural plants that are gaining popularity and acceptability in poultry nutrition. It has been determined that the performance of animals often increases with the addition of various phytobiotic additives to poultry feed, and that phytobiotics also show great effectiveness in protecting the normal beneficial microflora population while resisting pathogenic microorganisms in the intestine. Phytobiotics can be classified as herbs obtained from flowering, non-woody and non-permanent plants, botanicals or spices obtained from non-leaf parts such as seeds, fruits, barks or roots, essential oils and extracts and oleoresins. Many phytochemical-rich medicinal herbs are now evaluated as potential antimicrobial and growth promoter alternatives.Aromatic herbs have been successfully used in the poultry sector to increase antioxidant capacity Ginger and turmeric are among the dried plant roots used as phytobiotics in poultry feeding. In this study, we tried to give information about the studies in the last fifteen years on the use of ginger and turmeric, whose dried powdered roots are also used as tea, in the nutrition of broiler chickens.

GİRİŞ

Phytobiotics, which are phytochemical compounds obtained from plants, have been researched and widely used in animal nutrition in recent years as alternative feed additives to antibiotic growth promoters. Phytobiotics can be classified as herbs, botanicals derived from seeds, fruits, bark or roots of flowering, non-woody and non-permanent plants, or spices (having a strong odor or taste), essential oils or extracts, and oleoresins. Phytobiotics consist of natural bioactive compounds or substances of plant origin, including terpenoids, alkaloids, glycosides, and phenolics (Bote, 2004 , Shad et al., 2014). Numerous studies have been conducted to demonstrate the effect of phytobiotics as growth-promoting feed additives such as prebiotics and probiotics to improve the health conditions as well as the general performance parameters of poultry (Abd El-Ghany, 2020). Phytobiotics are also used as antimicrobial, antiparasitic, anticoccidial and immunostimulating agents in the poultry field (Hafeez et al., 2020). Phytobiotics are also called phytogenic or botanical. They are defined as natural, less toxic and residue-free plant-derived compounds used as feed additives in animal production (Wang et al., 2008). Each chemical class of phytobiotics has a mode of action. For example, phenolic compounds or flavonoids as major active ingredients are identified as potential antimicrobial and antioxidant agents. Therefore, the growth promotion results provided by phytobiotic feed additives vary depending on the phytochemical mixture used and their active chemicals and their concentrations (Rafeeq et al. 2023).

Ginger and turmeric are among the plants used as phytobiotics in poultry feeding. In this study, we tried to give information about the studies on the use of ginger and turmeric powder in broilers diets.

GINGER (*Zingiber officinale*)

Natural alternatives, such as medicinal and aromatic plants, have attracted attention due to their wide range of potential beneficial effects as alternatives to antibiotics. Ginger (Zingiber officinale) is an important crop grown mainly in Central Asia, China, India and Pakistan and exported worldwide. Ginger is widely used in traditional medicine to treat some ailments and as a spice. Ginger root contains various compounds with biological activities such as antioxidant, antimicrobial and pharmacological effects (Khan et al. 2012a). Ginger contains several active compounds including gingerol, shogaols, gingerdiol, and gingerdione. The main important compounds in ginger are gingerol, gingerdiol and gingerdione, which have the ability to stimulate digestive enzyme, influence microbial activity and have anti-oxidative activity. Ginger has been reported to have beneficial pharmacological chemicals due to its antioxidant, antibacterial, anti-inflammatory, antiseptic, anti-asiatic and immunomodulatory properties (Zhang et al. 2009, Eltazi, 2014). Ginger (Zingiber officinale) is the rhizome of the perennial plant Zingiber officinale, belonging to the Zingiberaceae family, and its root stem is widely used as a culinary spice and herbal medicine in many countries. Ginger is one of the plant-based additives used to increase the performance of poultry feed. Ginger powder has lipid-reducing effects and can also be used as a growth promoter. It has properties similar to antibiotics when included in poultry feed. These natural feed additives reduce enteric pathogen microbial loads and can increase performance by improving nutrient digestion and absorption (Abd El_Hack et al. 2020; Al-Khalaifah et al 2023). Preliminary research suggests that compounds found in ginger may bind to serotonin receptors, which may affect gastrointestinal function (Oleforuh-Okoleh et al. 2014).

The effects of using ginger powder (Zingiber officinale) in broiler diets were tried to be summarized (Table 1). Most researchers attributed the better performance of poultry fed with ginger to the improvement in the taste of this natural product, the presence of protease and lipase enzymes, and the rapid digestive effect (Irivboje et al. 2020; Abd El_Hack et al. 2020). The differences observed between studies were attributed to the different ginger types used, processing, dosage and trial period.

TURMERIC (*Curcuma longa)*

There have been grown interest in developing natural alternatives to antibiotic growth promoters in order to maintain both birds' performance and health. Turmeric has been extensively used in poultry diets. Turmeric is a natural herb of the ginger family, Zingiberaceae. Wide range medicinal properties of this plant have been advocated. In poultry feed, Turmeric has been extensively used in different concentrations, dosages and durations. Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae. It is native to tropical South Asia and requires temperature between 20 and 30°C and a considerable amount of annual rainfall for growth. The plant grows to a height of three to five feet and has oblong pointed leaves with funnel-shaped yellow flowers. The Curcuma longa extract is a yellow-orange poly-phenol and its usual form is a dry yellow powder that is oil-soluble in its natural state. The rhizome is the portion of the plant used medicinally (Khan et al 2012b). The active ingredients are tetrahydrocurcuminoids, curcumin, demethoxycurcumin bisdemethoxycutcumin.Curcumin (diferuloyl methane) the natural yellow pigment in the roots of Turmeric, is a poly-phenolic compound that is isolated from the rhizomes of Curcuma longa and related species (family Zingiberaceae). Curcumin, which gives yellow colour to Turmeric rhizomes, is one of the most active ingredients, responsible for the biological activity. Curcumin, which was found to have antioxidant, antibacterial activities, aflatoxin induced mutagenicity and hepatocarcinogenesis (Khan et al 2012b, Abou-Elkhair et al. 2014). Turmeric can act as an important source for an alternative to antibiotics due to its antimicrobial properties. It is less toxic, residue-free and natural compared to synthetic antibiotics or inorganic chemicals (Ali et al 2020). Studies have shown that addition of turmeric powder in broilers diets enhance their performance. However some research showed that 0.1 and 0.2% turmeric powder used as feed additive had no significant effects on the performance and carcass yield of broiler chickens (Fallah and Mirzaei, 2016).

The effects of using turmeric powder (*Curcumin longa*) in broiler diets were tried to be summarized (Table 2).

CONCLUSION

Many types of feed additives are used in broiler chicken rations to increase performance. It is quite common to use spices as additives in chicken diets. Plant active principles are chemical compounds found throughout the plant or in specific parts of the plant that provide therapeutic activity or beneficial effects. Adding spices and herbs to mixed feeds can provide many benefits to the health and performance of poultry, such as having anti-oxidative potential, antimicrobial activity, and improving digestion by stimulating endogenous enzymes. Ginger and turmeric are popular medicinal herb, which shows a wide range of pharmacological properties, such as antioxidant, antiangiogenic, antitumor, antivenom, antinflammatory, antimicrobial, antiproliferative, antiprotozoal, and antiaging. Many studies have been carried out with the inclusion of ginger and turmeric powder in the diet of broilers however the results have been quite different. The variation of results obtained in studies with ginger and turmeric in broiler performance can be explained by the variability in the amount of phytochemicals in the plants, since factors such as the age and stage of development of the plant, as well as the time when the harvest was performed, temperature, water availability, UV radiation, soil nutrients, altitude and atmospheric composition directly influence the relative proportions of these compounds in the plant. For this reason, before deciding to use medicinal aromatic plants in the nutrition of broiler chickens, the above-mentioned issues should be taken into consideration in order to obtain high efficiency from the broilers.

REFERENCES

Abd El-Ghany W.A., 2020. Phytobiotics in Poultry Industry as Growth Promoters, Antimicrobials and Immunomodulators -A Review. Journal of World Poultry Research, 10(4): 571-579.

Abd El-Hack, M. E., Alagawany, M., Shaheen, H., Samak, D., Othman, S. I., Allam, A. A., ... & Sitohy, M., 2020. Ginger and its derivatives as promising alternatives to antibiotics in poultry feed. Animals, 10(3), 452.

Abou-Elkhair, R., Ahmed, H. A., & Selim, S., 2014. Effects of black pepper (Piper nigrum), turmeric powder (Curcuma longa) and coriander seeds (Coriandrum sativum) and their combinations as feed additives on growth performance, carcass traits, some blood parameters and humoral immune response of broiler chickens. Asian-Australasian Journal of Animal Sciences, 27(6): 847-854.

Ahmed, I., El-Rayes, T., & Ahmed, A.I., 2018. Assessment of dietary supplementation of turmeric (curcuma longa) as a phytobiotic on broiler performance and bacterial count. Egyptian Journal of Nutrition and Feeds, 21 (2): 519-528.

Ali, M. Z., Islam, M. M., & Zaman, S., 2020. Effects of turmeric powder on Clostridium perfringens load in broiler chickens. SAARC Journal of Agriculture., 18(1): 209-218.

Al-Khalaifah, H., Al-Nasser, A., Al-Surrayai, T., Sultan, H., Al-Attal, D., Al-Kandari, R., ... & Dashti, F. (2022). Effect of ginger powder on production performance, antioxidant status, hematological parameters, digestibility, and plasma cholesterol content in broiler chickens. Animals, *12*(7), 901.

Bote, C.J.L., 2004. Bioflavonoid's effects reach beyond productivity. Feed Mix, 12: 12-15.

Darwısh,,M., and El- Shoukary, R., 2020. Effect of dietary supplementation of turmeric powder on growth performance, behavior and blood biochemical parameters of fayoumi broilers. Assiut Veterinary Medical Journal, 66(164), 15-23.

Eltazi, S., 2014. Response of Broiler Chicks to Diets Containing Different Mixture Levels of Garlic and Ginger Powder as Natural Feed Additives. International Journal of Pharmaceutical Research & Allied Sciences, 3(4):27-35.

Fallah, R., & Mirzaei, E, 2016. Effect of dietary inclusion of turmeric and thyme powders on performance, blood parameters and immune system of broiler chickens. Journal of Livestock Science, 7: 180-186.

Hafeez, A., Ullah, Z., Khan, R.U., Qudrat, U., and Naz, S., 2020. Effect of diet supplemented with coconut essential oil on performance and villus histomorphology in broiler exposed to avian coccidiosis. Tropical Animal Health and Production, 52: 2499-2504.

Irivboje, O. A., Olufayo, O., & Irivboje, Y.I., 2020. Phytogenic compounds: a review of ginger and garlic as an alternative feed additive in poultry nutrition. Proceedings of 25th Annual Conference of ASAN 2020, Abuja, Nigeria (299-302).

Khan, R. U., Naz, S., Javdani, M., Nikousefat, Z., Selvaggi, M., Tufarelli, V., & Laudadio, V. 2012b. The use of turmeric (Curcuma longa) in poultry feed. *World's Poultry Science Journal*, *68*(1), 97-103.

Khan, R. U., Naz, S., Nikousefat, Z., Tufarelli, V., Javdani, M., Qureshi, M. S., & Laudadio, V., 2012a. Potential applications of ginger (Zingiber officinale) in poultry diets. *World's* Poultry Science Journal, 68(2), 245-252.

Oleforuh-Okoleh, V. U., Chukwu, G. C., & Adeolu, A. I., 2014. Effect of ground ginger and garlic on the growth performance, carcass quality and economics of production of broiler chickens. Global Journal of Bioscience and Biotechnology, 3(3): 225-229.

Parvin, M. M., Beg, M. A. H., Begum, M., Islam, M. S., Rubel, M. Z. U., & Shafiqur, M., 2021. Effects of using garlic (Allium sativum) and turmeric (Curcuma longa) powder on production performance and biochemical parameters of broiler chicken. International Journal of Veterinary Science and Animal Husbandry, 6(4): 53-57.

Rafeeq, M., Bilal, R. M., Batool, F., Yameen, K., Farag, M. R., Madkour, M., ... & Alagawany, M., 2023. Application of herbs and their derivatives in broiler chickens: a review. World's Poultry Science Journal, 79(1): 95-117.

Shad, A.A., Ahmad, S., Ullah, R., Abd El-Salam, N.M., Fouad, H., Ur Rehman, N., Hussain, H., and Saeed, W., 2014. Phytochemical and biological activities of four wild medicinal plants. Scientific World Journal, 857363.

Shukla, U.K. and Kumar, P.V., 2018. Effect of turmeric (Curcuma longa L.) powder supplementation on growth performance of broiler chicks in cage system. Asian Journal of Animal Science, 13(2): 75-81.

Ürüsan, H., & Bölükbasi, Ş. C. (2017). Effects of dietary supplementation levels of turmeric powder (Curcuma longa) on performance, carcass characteristics and gut microflora in broiler chickens. JAPS: Journal of Animal & Plant Sciences, 27(3).

Wang, L., Piao, X.L., Kim, S.W., Piao, X.S, Shen, Y.B, and Lee, H.S., 2008. Effects of *Forsythia suspensa* extract on growth performance, nutrient digestibility, and antioxidant activities in broiler chickens under high ambient temperature. Poultry Science, 87: 1287-1294.

Zhang, G. F., Yang, Z. B., Wang, Y., Yang, W. R., Jiang, S. Z., & Gai, G. S.,2009. Effects of ginger root (Zingiber officinale) processed to different particle sizes on growth performance, antioxidant status, and serum metabolites of broiler chickens. Poultry Science, 88(10): 2159-2166.

USE OF CINNAMON AND CHAMOMILE IN BROILER DIETS

Taner İŞEVİ[1], Güray ERENER[1] Emrah GÜNGÖR[1], Şevket ÖZLÜ[1], Aydın ALTOP[1],

[1] Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Turkiye
Corresponding author e-mail: emrah.gungor@omu.edu.tr

ABSTRACT

Increasing bacterial resistance to synthetic antibiotics and consumer awareness of health and food safety concerns have triggered restrictions or bans on the use of antibiotic growth promoters in the poultry industry. Due to the restrictions on antibiotics in poultry nutrition, the poultry industry has turned to alternatives such as plant-derived feed additives. Thus, the poultry industry, focusing on developing more sustainable feed management strategies to improve the intestinal health and growth performance of poultry, has revealed phytogenic feed additives as natural alternatives to antibiotic growth promoters and encouraged research on this issue in poultry nutrition. Phytochemicals, plant bioactive compounds in poultry diets, have gained popularity due to their potential antioxidant and antimicrobial activities. These plants improve the immune system, reduce the stress response, and positively affect health and performance. Cinnamon bark and chamomile flower are among the plants used as phytobiotics in poultry feeding. In this study, we tried to give information about the studies in the last fifteen years on the use of cinnamon, whose bark is used as tea, and chamomile, whose flowers are used, in the nutrition of broiler chickens.

Key Words: Cinnamon, broiler, feed additives, nutrition

INTRODUCTION

In recent years, significant advances have been made to improve the health and performance of poultry. Feed is a major component of the poultry industry and exposes the gastrointestinal tract to a wide range of factors that can affect intestinal health. The gastrointestinal tract is considered a highly complex and dynamic organ that plays a crucial role in intestinal health (Ali et al. 2021). Various stress factors can negatively affect the balance in the intestinal ecosystem and ultimately the health status and productivity of poultry. Stress factors such as heat stress and gastrointestinal dysbiosis are considered major threats to the poultry industry due to their effects on intestinal health and increased disease susceptibility. The intestines of healthy animals can digest and absorb nutrients efficiently in environments where these stress factors are minimized (Diaz et al. 2019). Previously, antibiotic growth promoters were used to control gastrointestinal pathogens and reduce the effects of stress factors on intestinal function. However, increasing consumer awareness of the adverse effects of antibiotics on human health and concerns about increasing bacterial resistance and food safety have led to restrictions on the use of antibiotics in poultry production. This has led researchers and industry to look for alternatives to antibiotic growth promoters, focusing on developing more sustainable dietary interventions to improve the gut microbiome and overall health of poultry. Phytogenic feed additives have emerged as alternatives to antibiotic growth promoters and have great potential in the poultry industry (Ali et al .2021).

Cinnamon bark and chamomile flower are among the plants used as phytobiotics in poultry feeding. In this study, we tried to give information about the studies on the use of cinnamon, whose bark is used as tea, and chamomile, whose flowers are used, in the nutrition of broiler chickens.

CINNAMON (*Cinnamomum cassia)*

Cinnamon possess useful biological activities, including immunomodulatory, antioxidant, antiviral, lowering blood cholesterol, antimicrobial, lipid-lowering, antihypertension, anti-inflammatory, antitumor, gastroprotective, antidiabetic, neuroprotective and blood purifying properties. Dietary supplementation with cinnamon powder has been associated with positive effects on growth, digestion, antibacterial activities and immunity in chickens, as well as enhanced shelf life and quality of meat and reduced bacterial loading in eggs. On the basis of currently available literature, dietary supplementation levels with cinnamon has been variable for broilers (Saeed et al. 2018). In recent years, cinnamon, one of the most widely used spices, has attracted the attention of researchers as a natural product with numerous health benefits for poultry. Cinnamon belongs to the genus Cinnamomum (family Lauraceae), which includes more than 250-300 aromatic evergreen shrubs and herbs. However, only a few of these species, including Cinnamomum zeylanicum (C. zeylanicum: True Sri Lankan cinnamon), C. cassia (Cassia), C. burmanni (Indonesian cinnamon), and C. loureiori, are economically important worldwide as a common spice. has importance (Vietnamese cinnamon) (Ali et al., 2021). Cinnamon to poultry feed as a natural feed additive has beneficial effects on nutrient digestibility, hypocholesterolemia, blood biochemical profile, gene expression, immunity and especially intestinal health, alleviating the impact of disease and heat stress by maintaining water and electrolytic balance and feed intake. It has been clearly demonstrated that cinnamon can be used as an alternative to antibiotics in the poultry industry, offering greater animal welfare, food safety and economic aspects of poultry production (Ali et al., 2021). The main bioactive compounds of cinnamon are cinnamaldehyde, cinnamate and cinnamic acid, all of which play vital roles in various biological activities. It has been documented that the essential oil of cinnamon consists of natural antioxidant, anti-microbial and anti-inflammatory compounds such as flavonoids, curcuminoids, coumarins, tannins, alkaloids, xanthones, terpenoids, phenolics and other compounds (Purwanti, 2021). The concentration of volatile compounds in cinnamon essential oil depends mainly on the plant parts (leaves, bark, root, trunk) from which it is extracted). About forty-one volatile compounds have been identified in the bark oil of the cinnamon tree (Geng et al., 2011). Cinnamaldehyde (55% to 78%) is the main flavor compound in CNO extracted from bark while eugenol (59–78%) is the main compound in CNO that is extracted from leaves (Hamidpour et al. 2015). The volatile oil is approximately 0.6–1% and 1–2% phlobatannins, calcium oxalate, starch, mucilage, and mannitol in the bark (Ali et al., 2021). Use of cinnamon bark powder in the diet of broiler chicken at various level had generally positive impact on the performance in terms of BW, BWG, FI and FCR, overall performance index, carcass characteristics, blood profile and net profit per birds over (Chowlu et al. 2018, Gaikwad et al. 2020). The effects of using cinnamon bark powder (*Cinnamomum cassia*) in broiler diets were tried to be summarized (Table 1).

CHAMOMILLA (*Matricaria chamomilla* L.)

Chamamile flower (Matricaria chamomilla L.) has been a subject of considerable interest as a medicine and therapeutic agent throughout the world since ancient times. Flavonoids, sezekoitrepens a-bisabolol, chamazulene, farnesene and also sis and trans isomers of N-in dicyclotera are some prominent components of chamomile. Flavonoids are also encompassing of achi jinin, loteolin, koerestin, coumarin, ciranoz and patoliterin. This plant has aromatic flora with bitter taste. Its aroma is related to the camazolen essence and the bitter taste is attributed to some glycosides like apigenin and trithdroxy flavon. Chamomile is also containing phytoesterol and flavonoids like cyranosid and patulitrin (Dada et al. 2015). Chamomile (Matricaria chamomilla L.) is classified under a plant family composites. It contains flavones apigenin and essential volatile oil such as bisabol oxide B, "-bisabolo, chamazulene and bisaboloxide A. Chamomile flowers inhibit the excessive growth of harmful intestinal Micro organisms, thus counter acting inflammation it was reported that the addition of chamomile flowers at (0.25%) level to broiler diets improved growth performance and FCR (Al-Kaisse and Khalel, 2011). Including chamomile flowers are one of the medicinal plants used in the past and contain high levels of therapeutic compounds, namely bisabolol chamaxulene, beta-trans-farnesene, and flavone glycosides. Many bioactive phytochemical components were found in chamomile flower extract, including pentadecanoic acid, a palmitic acid methyl ester with antioxidant activity and -bisabolol (monocyclic sesquiterpene alcohol), which has anti-inflammatory, anti-bacterial, and anti-fungal properties (Tenorio et al. 2017). Anti-bacterial Chamomile also has positive effects on the digestive and respiratory systems and has an important role in boosting immunity and reducing oxidative stress (Alkado et al 2022). Chamomile flowers have antimicrobial and anti-inflammatory effects on broiler chicks, furthermore, it increased egg numbers of layers while decreased serum total cholesterol and GOT levels. Moreover, chamomile flowers improved growth performance and feed conversion ratio of broilers (Ahmed et al 2015). The effects of using chamamile flower powder (*Matricaria chamomilla L*) in broiler diets were tried to be summarized (Table 2).

CONCLUSION

Leaves, barks, flowers and essential oils of medicinal and aromatic plants are added to poultry feed to increase appetite, stimulate the secretion of endocrine and digestive enzymes, and stimulate antimicrobial, anti-inflammatory, antioxidative and immune activity. In this study, we tried to summarize the effects of using cinnamon bark and chamomile flower, especially dried forms, in the mixed feeds of broiler chickens. The place where the plants were grown, the type of the plant, the characteristics of the soil in which it was grown, weather conditions, altitude, season, harvesting method, storage conditions, phenolic substance contents, physiological states of the animals, growing conditions and experimental approaches may have been effective in obtaining different results from the studies examined. Cinnamon, in particular, can be toxic when used regularly and in high amounts due to the coumarin it contains, which has strong anticoagulant properties. For this reason, before deciding to use medicinal aromatic plants in the nutrition of broiler chickens, the above-mentioned issues should be taken into consideration in order to obtain high efficiency from the broilers.

REFERENCES

Ahmed, H.A., Sadek, K.M., & Taha, A.E., 2015. Impact of two herbal seeds supplementation on growth performance and some biochemical blood and tissue parameters of broiler chickens. International Journal of Animal and Veterinary Sciences, 9(3): 279-284.

Al-Abdullatif, A. A., Qaid, M. M., Al-Mufarrej, S. I., Al-Garadi, M. A., Murshed, M. A., & Alhotan, R. A. 2023. Impact of dietary supplementation with cinnamon bark powder on broiler growth and carcass indices, small intestinal measurements, physico-chemical properties of meat and economics of production. Cogent Food & Agriculture, 9 (2), 2284686.

Al-Hanna, R., Genova, K., & Al-Jammal, B., 2018. The impact of feeding with herbs Rosemary and Chamomile on broiler chickens body performance during the growth period. *Proceedings of the IX International Agricultural Symposium "Agrosym 2018" :*1730-1735.

Ali, A., Ponnampalam, E.N., Pushpakumara, G., Cottrell, J.J., Suleria, H.A.R., & Dunshea, F.R., 2021. Cinnamon: A Natural Feed Additive for Poultry Health and Production-A Review. Animals, 11, 2026.

Al-Kassie, G.A.M., 2010. The effect of thyme and cinnamon on the microbial balance in gastro intestinal tract on broiler chicks. International Journal of Poultry Science, 9 (5):495-498.

Chowlu, H., Vidyarthi, V. K., Zuyie, R., & Maiti, C. S., 2019. Use of cinnamon in diet of broiler chicken-A Review. Livestock. Research International, 6: 42-47.

Diaz Carrasco, J.M., Casanova, N.A., & Fernández Miyakawa, M.E., 2019. Microbiota, Gut Health and Chicken Productivity: What Is the Connection? Microorganisms, 7:374.

Gaikwad, D., Sharma, N. R., Prabhakar, P. K., & Sıngh, J., 2020. Application of ginger and cinnamon in poultry nutrition on growth performance: A Review. Plant Cell Bıotechnology And Molecular Bıology, 21 (71-72): 172-180.

Geng, S., Cui, Z., Huang, X.; Chen, Y.; Xu, D., & Xiong, P., 2011.Variations in essential oil yield and composition during Cinnamomum cassia bark growth. Industrial Crops and Products. 33: 248-252.

Hamidpour, R., Hamidpour, M., Hamidpour, S., & Shahlari, M., 2015. Cinnamon from the selection of traditional applications to its novel effects on the inhibition of angiogenesis in cancer cells nd prevention of Alzheimer's disease, and a series of functions such as antioxidant, anticholesterol, antidiabetes, antibacterial, antifungal, nematicidal, acaracidal, and repellent activities. Journal of Traditional and Complementary Medicine 5: 66-70.

Kanani, P. B., Daneshyar, M., Aliakbarlu, J., & Hamian, F., 2017. Effect of dietary turmeric and cinnamon powders on meat quality and lipid peroxidation of broiler chicken under heat stress condition. In Veterinary Research Forum 8(2):163-169.

Khishtan, A.T.M., & Beski, S.S.M., 2020. Delivery route of chamomile on the growth and subsequent physiology of broiler chickens under E. coli challenge. The Iraqi Journal of Agricultural Science, 51(4):1058-1073.

Mahmmod, Z.A., 2013. The effect of chamomile plant (matericaria chamomile L.) as feed additives on productive performance, carcass characteristics and immunity response of broilers. International Journal of Poultry Science, 12 (2): 111-116.

Nassar, H., Abdel-Raheem, H., & Sotohy, S., 2019. Effect of dietary olive leaves and chamomile flowers powder on the growth performance and carcass traits and of broilers (Sasso Breed). Assiut Veterinary Medical Journal, 65(163), 58-67.

Odutayo, O. J., Adeyemo, A. A., Ibigbami, D. J., Sogunle, O. M., Olaifa, R. O., Akinwale, O. A., ... & Joel, A.O., 2021. Influence of cinnamon (Cinnamomum cassia) powder as additive on jejenal histomophometry, growth performance, haemato-biochemical indices, carcass traits and breast meat lipid profile of broiler chickens. Nigerian Journal of Animal Production, 48(6): 167-184.

Purwanti, S. 2021. The functions of Cinnamomum burmannii as an antioxidant feed additive for broiler chickens: A review. In *IOP Conference Series: Earth and Environmental Science* (788(1): 012082). IOP Publishing.

Qaid, M. M., Al-Mufarrej, S. I., Azzam, M. M., Al-Garadi, M. A., Alqhtani, A. H., Al-Abdullatif, A. A., ... & Suliman, G.M., 2022. Dietary cinnamon bark affects growth performance, carcass characteristics, and breast meat quality in broiler infected with Eimeria tenella oocysts. Animals, 12 (2): 166.

Saeed, M., Kamboh, A. A., Syed, S. F., Babazadeh, D., Suheryani, I., Shah, Q. A., ... & Chao, S., 2018. Phytochemistry and beneficial impacts of cinnamon (Cinnamomum zeylanicum) as a dietary supplement in poultry diets. World's Poultry Science Journal, 74(2), 331-346.

Tenorio, K.I., Sgavioli, S., Roriz, B.C., Ayala, C.M., Santos, W.d., Rodrigues, P.H.M., Almeida, V.R.d., and Garcia, R.G., 2017. Effect of chamomile extract on the welfare of laying Japanese quail, Revista Brasileira de Zootecnia, 46 (9): 760-765.

Toghyani, M., Ghasemi, A., & Tabeidian, S.A., 2015. The effect of different levels of seed and extract of harmal (Peganum harmala L.) on immune responses of broiler chicks. International Journal of Animal and Veterinary Sciences, 9(1): 51-54.

STUDY OF THE MARE'S ESTRUS BEHAVIOUR INTENSITY DURING THE BREEDING SEASON

Amel NAJJAR[1], Siwar NJOUMI[1], Afef ISMAIL[2], Salama BEN ACHOUR[2], Soffien EZZAR[2], Belgacem BENAOUN[2], Sana KHALDI[3]

[1] Department of Animal Sciences, National Institute of Agronomy of Tunisia, University of Carthage, Tunisia.
[2] National Foundation of the Horse Breed Improvement, Tunisia, National Foundation of the Horse Breed Improvement, Tunisia.
[3] National School of Veterinary Medecine of Sidi Thabet, University of La Manouba, Tunisia.

Corresponding author e-mail: amel.najarbenmaatou@inat.ucar.tn

ABSTRACT

The present study aimed to diagnosis the mare's sexual activity during the breeding season. One hundred and seventy (170) of Arabian mares, aged between 04-27 years and reared in the National stud farm of Sidi Thabet, were followed during the official breeding season, from February to May 2022, while they were submitted to a natural mating (NM) service. Estrus monitoring was carried out daily through a teaser test, and if positive, the female went through an ultrasound examination starting at the 3rd day of estrus for the follow up of the follicular activity. During the estrus period, the behavioral expression in females was assessed and its intensity recorded, then the natural mating was performed every 48 hours as soon as the ovulatory follicular diameter reached 35 mm, and stopped when the corpus luteum was detected. Mares with a delay of resumption of ovarian activity or with cyclicity problems were treated with hormonal injections. The results showed that mares showing a clear estrus behaviour during the breeding season could be distributed into a typology, represented as following: i) according to their age, 78% of mares were older and 22% were young, ii) according to their status, 88% of mares were multiparous (barren and mares with foal, 41% and 47% respectively) versus the maiden ones (12%). A total of 26% of the observed mares showed a problem of resumption of ovarian activity and did not show any estral behaviour, and were thus treated with GnRH (14%) and prostaglandin F2a (12%). The average estrus and diestrus duration were 8.2 and 13.8 days, respectively. The estrus behaviour intensity varied during the breeding season and was higher during March and April, and started to decrease in May ($p<0.05$). Estrus behaviour intensity was weak in foaling estrus compared to the cyclic estrus. The results suggested that the estrus behaviour intensity depends on the mare's type estrus and tends to decrease at the end of the breeding season. The weak estrus intensity shown in mares during their foaling estrus and at the end of the breeding season leads to mare's rigorous monitoring of their follicular activity using ultrasonography in order de secure pregnancy.

Keywords: sexual activity, estrus intensity, breeding season, Arabian mares.

INTRODUCTION

Estrus behaviour intensity in mare can vary depending on the individual and the hormonal levels (Stachurka et al., 2023). Some common signs of estrus behaviour include increased vocalization, restlessness, frequent urination, tail flagging, and receptivity to stallion (McDonnel, 2000). Mares may also show signs of aggression or irritability during estrus. It is

important to horse owners and handlers to be aware of these behaviours so they can properly manage and handle the mare during this time. Additionally, it is important to monitor the mare's behaviour and consult with a veterinarian if there are any concerns about her estrus behaviour intensity.
The objective of the study aimed to diagnosis the mare's sexual activity during the breeding season.

MATERIAL AND METHODS

The study took place in the National Stud Farm of Sidi Thabet of the National Foundation of the Improvement of the Horse Breed (FNARC), in the North of Tunisia, at 24 km from Tunis. The experiment involved 170 Arabian Mares aged between 4 and 27 years, and were followed during the official breeding season, from February to May 2022, while they were submitted to a natural mating (NM) service.

Estrus' mare monitoring was carried out daily through a teaser test in the breeding courtyard, and if it was positive, the female went through an ultrasound examination starting at the 3rd day of estrus for the follow up of the follicular activity: The mare is placed in 'restraint work' to prevent possible accidents and facilitate handling. The mare is immobilised with a twisted nose in order to free the vulvo-perineal area. After emptying the rectum of faeces, transrectal palpation is carried out to get an idea of the size and consistency of the various parts of the female's genital tract and to locate the position of the ovaries. The ultrasound examination of the ovaries was carried out using an ALOKA ultrasound scanner (SSD 500) equipped with a 5 MHz linear recto-dynamic probe (model UST-587I-5) (Figure 1). The gel-lubricated probe is inserted into the mare's rectum. It is then pointed at the cervix, the uterine body, the right or the left uterine horn and finally the right or the left ovary to visualize follicular activity. If the mare is in heat, the evolution of follicular development; the diameter of the pre-ovulatory follicle is determined. If the mare is not in heat, the corpus luteum is noticed on the ovary. This shows that ovulation is completed (Haras Nationaux, 2004).
The follicular activity of mares in natural service is monitored daily until the pre-ovulatory follicle reaches 35mm in diameter. At this point, the mare is serviced every 48 hours until the corpus luteum is detected by ultrasound.

Figure 1. Ultrasound monitoring of follicular activity in mare.

During the estrus period, the behavioral expression in females was assessed and its intensity was recorded, then the natural mating was performed every 48 hours as soon as the ovulatory follicular diameter reached 35 mm, and stopped when the corpus luteum was detected. Mares with a delay of resumption of ovarian activity or with cyclicity problems were treated with hormonal injections (GnRH: Receptal® and prostaglandin F2a: Estrumate®).
Statistical analysis was carried out using SAS software (SAS Institute Inc., Cary, NC, USA). The General Linear Model (GLM) procedure was used to study the influence of the breeding season months on the estrus sign behaviour intensity in mares. The means were compared using the SNK test, and the level of signification was fixed at $p < 0.05$.

RESULTS AND DISCUSSION

The results showed that mares showing a clear estrus behaviour during the breeding season could be distributed into a typology, represented as following: According to their age (Figure 2), 78% of mares that exhibit estrus signs were older and 22% were young.

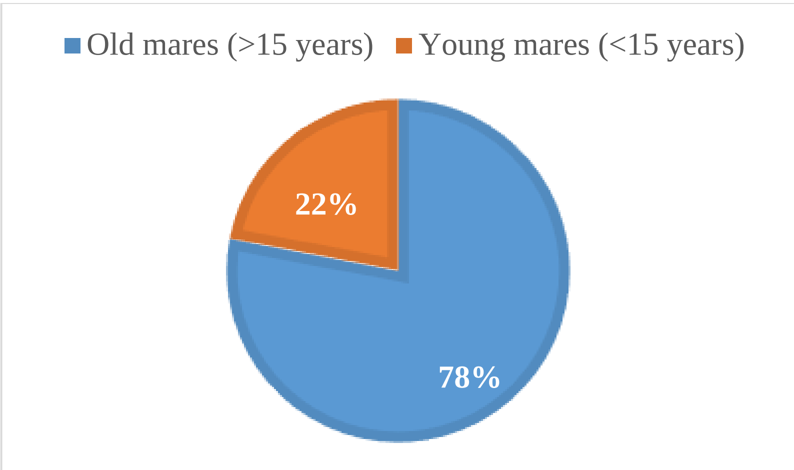


Figure 2. Variation of the percentage of mares that exhibit estrus behaviour according their age.

The intensity of estrus behaviour in mares over 15 years of age did not differ significantly ($p>0.05$) from that in mares under 15 years of age. This suggests that age does not affect the intensity of heat behaviour. This result is in line with that reported by El Ghali (2010), who proved that age has no effect on estrus behaviour in mares. However, according to Crowell-Davis (2007), young mares may exhibit an oestrus that is less attractive to the stallion than older mares.
According to their status (Figure 3), 88% of mares that exhibit estrus behaviour were multiparous (barren and mares with foal, 41% and 47% respectively) versus the maiden ones (12%).

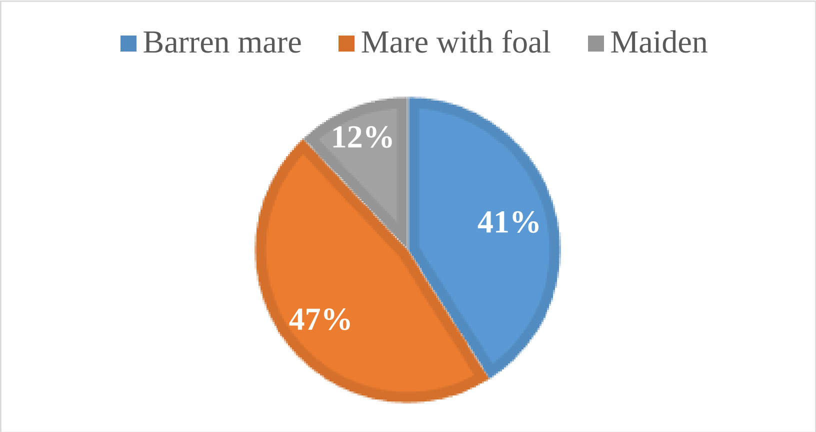


Figure 3. Variation of the percentage of mares that exhibit estrus behaviour according their status.

A total of 26% of the observed mares showed a problem of resumption of ovarian activity and did not show any estral behaviour (Table 1), and were thus treated with GnRH (14%) and prostaglandin F2a (12%).

Table 1. Percentage of mares requiring hormonal estrus induction treatment.

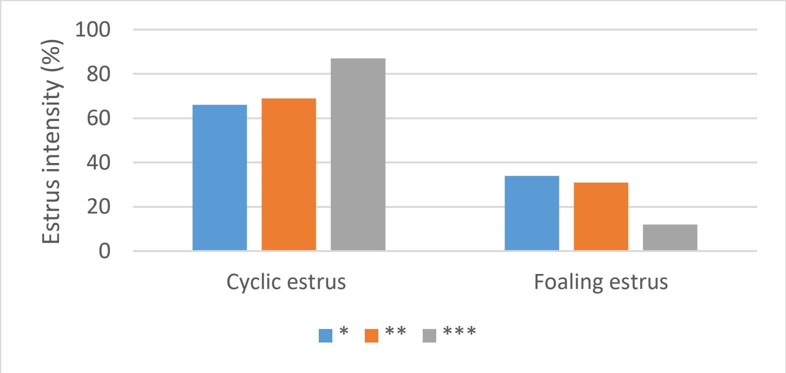
	Normal ovarian resumption (%)	Problem of ovarian resumption which involved estrus induction	
		GnRH treatment (%)	Prostaglandin F2a treatment (%)
		14	12
Total	74	26	

The average estrus and diestrus duration were 8.2 and 13.8 days, respectively, and the average estrus cycle duration and the overall mean of the ovulatory follicle were 21.8 days and 38.7 mm, respectively (Table 2).

Table 2. Variation of overall means of estrus cycle, estrus, diestrus and ovulatory follicle diameter.

	Means	Standard deviation	Min	Max
Average duration of estrous cycle (days)	21.83	3.22	17	27
Average duration of the estrus (days)	8.20	2.10	4	12
Average duration of dioestrus (days)	13.77	2.82	7	19
Overall average ovulatory follicle diameter (mm)	38.73	6.66	23	64

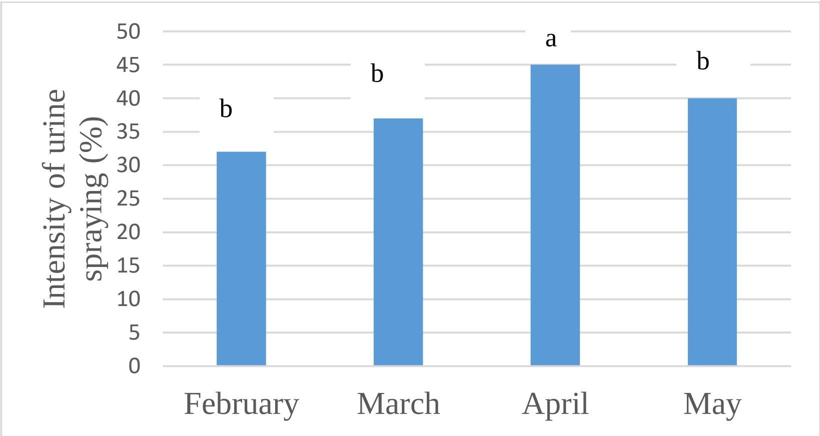
Estrus behaviour intensity was weak in foaling estrus compared to the cyclic estrus (Figure 4). The sign of estrus behaviour in the camped position was more visible and intense in mares in cyclical estrus than in those in foaling estrus. According to Meadows et al (2003) and Coleman and Powel (2004) the estrus behaviour of a mare that has foaled can be masked by her maternal behaviour.



: weak intensity of estrus, **: moderate intensity of estrus, *: strong intensity of estrus*

Figure 4. Variation the estrus behaviour intensity in the camped position in both cyclic and foaling estrus.

The estrus behaviour intensity varied during the breeding season and was higher during April, and started to decrease in May (Figure 5; $p<0.05$). The sign of urine spraying followed an ascending trend starting with a percentage of 32% in February, 37% in March and a peak in April (45%), before decreasing again in May (40%).
According to Asa (2007) oestrus behaviour in mares involves an increasing intensity of urine spraying as a function of time. This can be explained by the fact that as the mare progresses through the oestrus stage, she camps and urinates without being approached by the stallion teaser. The absence and the low levels of intensity recorded in May can be explained by the mare's seasonal sexual activity, which begins to deteriorate towards the end of the breeding season, and by the environmental conditions in Tunisia, particularly the photoperiod and the temperature.



a,b : p<0.05

Figure 5. Variation of the estrus behaviour intensity of urine spraying in both cyclic and foaling estrus.

CONCLUSIONS

The results suggested that the estrus behaviour intensity depends on the mare's type estrus and tends to decrease at the end of the breeding season. The weak estrus intensity shown in mares during their foaling estrus and at the end of the breeding season leads to mare's rigorous monitoring of their follicular activity using ultrasonography in order to secure pregnancy.

REFERENCES

Asa C.S., 2007. Equid reproductive biology: 113-117.

Crowell-Davis S.L., 2007. Sexual behaviour of mares. Elsevier Science. Hormones and Behavior 52: 12-17.

El Ghali I., 2010. Etude des facteurs de variation de la fertilité de la jument Pur-Sang Arabe au Haras National de Sidi Thabet. Mémoire de mastére, INAT, Tunis, p89.

Haras Nationaux, 2004.

McDonnell S.M., 2000. Reproductive behavior of stallions and mares: Comparison of free-running and domestic in-hand breeding. *Anim. Reprod. Sci.*,60–61:211–219.

Meadows, D.G., Hopkins, F.M., Henton, J.E., 2003. Heat detection and teasing systems for mares. Departement of Large Animal Clinical Science, College of Veterinary Medicine.

Stachurska A., Kędzierski W., Kaczmarek B., Wiśniewska A., Żylińska B., Janczarek I., 2023. Variation of Physiological and Behavioural Parameters during the Oestrous Cycle in Mares. Animals, 13(2):211.

INVESTIGATION OF SCION GROWTH SITUATION OF SOME PEACH VARIETIES GRAFTED ON DIFFERENT CLONAL ROOTSTOCKS

Remzi Uğur[1*], Mehmet Ali Sarıdaş[2], Halit Seyfettin ATLI[3]

[1]Gaziantep University Nurdagi Vocational School
[2]Cukurova University, Faculty of Agriculture, Department of Horticulture
[3]Siirt University Faculty of Agriculture Department of Horticulture
[*] Corresponding author, E-mail: remzibey@hotmail.com

ABSTRACT

In rootstock breeding studies, examining the effects of rootstock on the growth vigor of varieties is an important stage. It is known that rootstocks are effective in resistance to soil and climatic conditions and growth vigor in fruit trees. The study was carried out in the greenhouses of the Eastern Mediterranean Transitional Zone Agricultural Research Institute. The study examined the effects of 33 selected clone rootstocks and 4 standard control rootstocks on plant development in Transvalia and Flored peach varieties. In this study, it was determined that rootstocks have significant effects on plant growth vigor in peach varieties. As rootstock scion combinations, it was determined that the highest rootstock diameter development was in Gn-22 (15.41 mm) and the lowest rootstock diameter development was in KL-26 (9.25 mm). In scion diameter developments, the highest was in Gn-22 (13.36 mm) and the lowest was in KL-4 (7.86 mm) rootstocks. The study observed that the Flored variety was stronger than the Transvalia variety in terms of growth vigor.

Key Words: Rootstock candidates, graft compatibility, seedling development, selection breeding

INTRODUCTION

Peach [Prunus persica (L.) Batsch] is the second most widely cultivated fruit species in the world after apples. In modern peach cultivation, selecting varieties and rootstocks suitable for climate and soil conditions is very important (Reig et al. 2016). In recent years, we have seen the breeding of many peach and nectarine varieties. The accelerated variety breeding efforts have led to the introduction of various new varieties, attracting increasing interest from consumers and producers. This situation necessitates accelerating rootstock breeding efforts suitable for these newly developed varieties (Iglesias, Carbo, and Bonany 2009). A few standard rootstocks are used in the newly bred peach and nectarine varieties. In fact, it would be more appropriate to use new rootstocks that are more compatible with these varieties and positively affect yield and quality according to the characteristics of the variety (Jimenez et al. 2011). Various rootstock breeding programs have been conducted to develop rootstocks with different characteristics for use in stone fruit cultivation (Moreno, Tabuenca, and Cambra 1994; Felipe et al. 1997; Beckman and Lang 2002; Loreti and Massai 2002). Rootstocks can be considered a technical tool in modern fruit growing for increasing fruit yield and quality, ensuring the plant's resistance to abiotic conditions, and controlling growth vigor to adjust planting density (Lopez-Ortega et al. 2016; Reig et al. 2018a). Therefore, selecting the right rootstock for economically efficient peach orchards in high-density plantings, high pH calcareous or sandy soils, and areas where nematode and root rot are problems becomes important (Reighard and Loreti 2008). In most Mediterranean coastal countries, peach-almond hybrids (P. amygdalus P. persica) are widely used as rootstocks for peach trees. These rootstocks, tolerant of high pH alkaline soils, are also resistant to iron chlorosis. Peach-almond hybrid rootstocks also form compatible combinations with peach varieties grafted onto them (Zarrouk et al. 2005). In recent years, new peach-almond hybrid rootstocks resistant to root-knot nematodes (Meloidogyne spp.) have also been developed (Pinochet 2009). Similarly, it has been reported that plum-derived rootstocks have been developed and marketed for peaches and other stone fruits (Moreno, Tabuenca, and Cambra 1995). Plum-derived rootstocks adapt well to very calcareous and heavy-textured soils, are tolerant to root asphyxia and iron chlorosis, and are resistant to root-knot nematodes. These rootstocks are selected for peaches for dwarf growth and high-density orchard establishment, strong graft compatibility, sustainable productive and quality fruit production (Reighard and Loreti 2008). The adoption of high-density orchards in fruit growing, obtaining higher yields per unit area, and the widespread cultivation of early peaches, including under cover, may be possible with the use of less vigorous or dwarf rootstocks. However, a satisfactory rootstock with characteristics suitable for these conditions and compatible with stone fruits has not yet been reached (Ben Yahmed, Ghrab, and Ben Mimoun 2016; Ben Yahmed et al. 2016). Studies have reported that rootstocks affect fruit yield and quality, tree health, the uptake of water and mineral substances from the soil, leaf gas exchange, flowering, formation of high-quality fruit buds, and harvest date (Font i Forcada, Gogorcena, and Moreno 2014; Ben Yahmed, Ghrab, and Ben Mimoun 2016; Reig et al. 2018a). Rootstocks also affect the mineral content of the leaves and the uptake of mineral nutrients (Zarrouk et al. 2005; Mestre et al. 2015). For the successful production of deciduous fruit species, selecting an appropriate graft combination appears to be very important. Because the interaction between rootstock and scion also affects water relations, leaf gas exchange, and plant growth vigor (Gonçalves et al. 2003). Various hypotheses have been proposed to explain the effects of rootstocks on grafted varieties. According to these hypotheses, the relationship of plant nutrients/water uptake from the soil and the interaction of hormones produced in the plant's underground and aboveground parts significantly affect the fruit yield-quality, growth form, and vigor of that tree (Sorce et al. 2002; Solari and DeJong 2006; Solari, Pernice, and DeJong 2006).

This study was planned and conducted to explain the effects of the rootstock-scion relationship on plant growth vigor in the "Transvalia" peach variety. The study was carried out in the greenhouse and field of the Eastern Mediterranean Transitional Zone Agricultural Research Institute in 2019-2020.

MATERIALS AND METHODS

The materials of the study consisted of 33 clonal rootstocks belonging to the genus Prunus obtained through selection and hybrid breeding (Table 1). The Transvalia and Flored peach varieties were grafted onto the selected and control rootstocks within the scope of the trial. In the study, the rootstock and scion diameters of these apricot varieties grafted onto selected rootstocks were measured with a caliper, and the seedling heights were measured with a meter at 15-day intervals from May to October. Statistical analysis was applied to the values at the end of the vegetation period in which the trial was conducted. Thus, the effects of rootstocks, varieties, and their interactions on rootstock diameter, scion diameter, and seedling height were tried to be observed.

The study was designed as a randomized block design, with three replications and five plants per replication. The obtained data were analyzed using analysis of variance ($P<0.05$). The comparison of means was carried out using the LSD test.

Table-1. Rootstock candidates found promising in tissue culture studies, with grafting and seedling development performances under investigation.

No	Origin of Rootstock	Clon
1	*P.cerasifera*	KL-29
2	*P.cerasifera*	KL-45
3	*P.cerasifera*	KL-59
4	*P.cerasifera*	KL-38
5	*P.divaricata*	KL-46
6	*P.cerasifera*	KL-57
7	*P.divaricata*	KL-47
8	*P.cerasifera*	KL-58
9	*P.domestica*	KL-6
10	*P.cerasifera*	KL-60
11	*P.domestica*	KL-1
12	*P. amygdalus x P. cerasifera*	FC118
13	*P. amygdalus x P. cerasifera*	FS-148
14	*P. amygdalus x P. cerasifera*	FS-185
15	*P.divaricata*	KL-5
16	*P.domestica*	KL-30
17	*P.domestica*	KL-44
18	*P. amygdalus x P. cerasifera*	FS-197
19	*P. amygdalus x P. cerasifera*	FS-1
20	*P.domestica*	KL-26
21	*P. amygdalus x P. cerasifera*	FS-141
22	*P. amygdalus x P. cerasifera*	FC22
23	*P. amygdalus x P. cerasifera*	FC83
24	*P.divaricata*	KL-4
25	*P. amygdalus x P. cerasifera*	FC19
26	*P. amygdalus x P. cerasifera*	FC44
27	*P. amygdalus x P. cerasifera*	FC48
28	*P. amygdalus x P. cerasifera*	FC73
29	*P. amygdalus x P. cerasifera*	FC76
30	*P. amygdalus x P. cerasifera*	FS-43
31	*P. amygdalus x P. cerasifera*	FS-86
32	*P. amygdalus x P. cerasifera*	FS-184
33	*P. microcarpa x P. cerasifera*	PCXPM4

RESULTS

The rootstock diameter values for the Flored and Transvalia peach varieties grafted onto selected and control rootstocks at the end of the vegetation period of the study are presented in

Table 10. When examining this parameter, it was found that the differences among rootstocks, varieties, and the rootstock x variety interactions were statistically significant. The rootstock diameter values ranged from 9.07 mm to 15.41 mm. The highest rootstock diameter values were observed in GN-22 (15.41 mm), KL-38 (15.28 mm), and GF-677 (15.24 mm), and these rootstocks were in the same statistical group. The lowest rootstock diameter values were measured as 9.07 mm and 9.25 mm for KL-4 and KL-26, respectively. The rootstock diameter values of the varieties were close to each other, with Flored at 12.47 mm and Transvalia at 12.22 mm. The 0.25 mm difference was considered significant. Among the rootstock x variety interactions, the highest rootstock diameter values were obtained in the GN-22/Flored (15.56 mm), KL-38/Flored (15.44 mm), and GF-677/Flored (15.39 mm) combinations.

The scion diameter values for the Flored and Transvalia peach varieties grafted onto selected and control rootstocks at the end of the vegetation period of the study are presented in Table 11. It was found that the differences among the two factors examined and their interactions were statistically significant. The scion diameter values ranged from 7.86 mm to 13.36 mm. The highest scion diameter values were found in GN-22 (13.36 mm), KL-38 (13.26 mm), and GF-677 (13.22 mm), with no significant statistical differences among these rootstocks. The lowest scion diameter values were measured as 7.86 mm and 8.02 mm for KL-4 and KL-26, respectively. The effects of the varieties on scion diameter values were close to each other, with the Flored variety measuring 10.81 mm and the Transvalia variety measuring 10.60 mm. In the rootstock x scion interactions, the highest scion diameter values were obtained from the GN-22/Flored (13.50 mm), KL-38/Flored (13.39 mm), and GF-677/Flored (13.35 mm) combinations.

The seedling height values for the Flored and Transvalia peach varieties grafted onto selected and control rootstocks at the end of the vegetation period of the study are provided in Table 12. It was found that the differences among the two factors investigated and their interactions were statistically significant. The seedling height values were observed to be similar to the rootstock and scion values. The highest seedling height values were measured as 138.47 cm in GN-22 rootstock. This rootstock was followed by GF-677 and KL-38 rootstocks, and these three rootstocks were in the same statistical group. The lowest seedling height was measured as 81.47 cm in KL-4 rootstock. Among the rootstocks examined in the study, 5 produced seedlings shorter than 1 meter. The seedling heights for varieties were close to each other, with Flored at 112.05 cm and Transvalia at 109.86 cm. In the rootstock x scion interactions, the highest seedling height values were observed in GN-22/Flored (139.84 cm), KL-38/Flored (138.70 cm), and GF-677/Flored (138.32 cm) combinations.

Table 10. Rootstock diameter values (mm) in peach varieties grafted onto selected and controls

Rootstock	Variety		Mean of Rootstock
	Flored	Transvalia	
KL-38	14.44 a	15.13 a	**15.28 A**
KL-46	13.74 b-e	13.47 d-g	**13.60 CD**
KL-29	14.16 b	13.88 bcd	**14.02 B**
KL-45	13.67 b-f	13.40 d-g	**13.53 CD**
KL-47	12.78 ı-n	12.53 k-p	**12.65 GH**
KL-58	12.35 r-q	12.11 p-v	**12.23 IJK**
KL-57	12.59 j-p	12.34 m-r	**12.46 HI**
KL-60	12.62 j-o	12.37 l-q	**12.49 HI**
KL-59	12.85 h-l	12.59 j-p	**12.72 GH**
KL-30	11.37 zA	11.14 A	**11.25 O**
KL-44	10.34 A	10.14 A	**10.24 P**
FS-1	13.57 c-f	13.30 e-h	**13.43 DE**
FS-197	13.98 bc	13.70 b-e	**13.84 BC**
FS-141	13.44 d-g	13.17 f-ı	**13.30 DE**
FS-185	13.29 e-h	13.03 g-k	**13.16 EF**
FS-148	13.70 b-e	13.43 d-g	**13.56 CD**
FS-184	11.96 q-x	11.72 t-z	**11.8r LMN**
FC-44	12.52 l-p	12.28 n-s	**12.40 HI**
FC-73	12.40 l-q	12.16 o-t	**12.58 IJ**
FS-86	11.84 r-z	11.61 v-A	**11.69 LMN**
FC-76	11.81 s-z	11.58 w-A	**11.69 LMN**
KL-5	13.06 g-j	12.81 h-m	**12.93 FG**
FC-19	11.90 q-y	11.66 t-z	**11.78 LMN**
KL-6	9.63 A	9.44 AB	**9.53 Q**
KL-26	9.34 A-D	9.16 AB	**9.25 QR**
FC-48	12.16 o-t	11.92 q-y	**12.04 JKL**
FS-43	11.70 t-z	11.47 x-A	**11.58 MNO**
FC-118	11.64 u- A	11.42 yzA	**11.53 NO**
FC-22	12.13 o-u	11.90 q-y	**12.01 JKL**
KL-1	9.54 A	9.35 AB	**9.44 Q**
KL-4	9.16 A-D	8.98 B	**9.07 R**
FC-83	13.71 b-e	13.44 d-g	**13.57 CD**
PCPM4	11.61 v - A	11.38 zA	**11.49 NO**
GF-677	15.39 a	15.09 A	**15.24 A**
R-20	12.01 q-w	11.77 t-z	**11.89 KLM**
GN-22	15.56 a	15.26 a	**15.41 A**
Mean of Variety	**12.47 A**	**12.22 B**	
LSD	**Rootstock: 0.35****	**Variety: 0.08****	**RootstockxVariety: 0.49****

1-Different letters indicate that differences between means. In the interaction part, uppercase letters used after lowercase letters when comparing means. Rootstock means written in bold uppercase letters, variety means written in bold italic uppercase letters. 2- N.S.: Not Significant; **: p<0.01; *<0.05.3- Numbers in parentheses are arc transformation values.

Table 11. Scion diameter values (mm) in peach varieties grafted onto selected and control rootstocks (mm)

Rootstock	Variety		Mean of Rootstock
	Flored	**Transvalia**	
KL-38	13.39 a	13.13 a	**13.26 A**
KL-46	11.91 b-e	11.68 d-g	**11.79 CD**
KL-29	12.28 b	12.04 bcd	**12.16 B**
KL-45	11.85 b-f	11.62 d-g	**11.53 CD**
KL-47	11.08 ı-n	10.87 k-p	**10.97 GH**
KL-58	10.71 l-r	10.50 p-x	**10.60 IJK**
KL-57	10.92 j-p	10.70 m-s	**10.81 HI**
KL-60	10.95 j-o	10.73 l-r	**10.84 HI**
KL-59	11.14 h-l	10.92 j-o	**11.03 GH**
KL-30	9.86 BC	9.86 BC	**9.76 O**
KL-44	8.97 D	8.79 D	**8.88 P**
FS-1	11.77 c-f	11.54 e-h	**11.65 DE**
FS-197	12.12 dc	11.89 b-e	**12.00 BC**
FS-141	11.65 d-g	11.42 f-ı	**11.53 DE**
FS-185	11.52 e-h	11.30 g-k	**11.41 EF**
FS-148	11.88 b-e	11.65 d-g	**11.76 CD**
FS-184	10.37 q-z	10.17 u-B	**10.27 LMN**
FC-44	10.86 l-p	10.65 n-t	**10.75 HI**
FC-73	10.76 l-q	10.55 o-u	**10.65 IJ**
FS-86	10.27 s-B	10.07 x-C	**10.17 LMN**
FC-76	10.25 t-B	10.05 y-C	**10.15 LMN**
KL-5	11.33 g-j	11.11 h-m	**11.22 FG**
FC-19	10.32 r-A	10.11 v-B	**10.21 LMN**
KL-6	8.35 E	8.19 EF	**8.27 Q**
KL-26	8.10 EF	7.95 EF	**8.02 QR**
FC-48	10.54 o-v	10.37 q-z	**10.44 JKL**
FS-43	10.14 u-B	9.95 z-C	**10.04 MNO**
FC-118	10.10 w-B	9.90 ABC	**10.00 NO**
FC-22	10.52 o-w	10.32 r-A	**10.42 JKL**
KL-1	8.27 EF	8.11 EF	**8.19 Q**
KL-4	7.94 EF	7.79 EF	**7.86 R**
FC-83	11.89 b-e	11.66 d-g	**11.77 CD**
PCPM4	10.07 x-C	9.87 BC	**9.97 NO**
GF-677	13.35 a	13.09 a	**13.22 A**
R-20	10.41 q-y	10.21 u-B	**10.31 KLM**
GN-22	13.50 a	13.23 a	**13.36 A**
Mean of Variety	**10.81 A**	**10.60 B**	
LSD	**Rootstock: 0.29****	**Variety: 0.06****	**RootstockxVariety: 0.43****

1-Different letters indicate that differences between means. In the interaction part, uppercase letters used after lowercase letters when comparing means. Rootstock means written in bold uppercase letters, variety means written in bold italic uppercase letters. 2- N.S.: Not Significant; **: p<0.01; *<0.05.3- Numbers in parentheses are arc transformation values.

Table 12. Seedling height values (cm) in peach varieties grafted onto selected and control rootstocks (cm)

Rootstock	Variety		Mean of Rootstock
	Flored	Transvalia	
KL-38	138.70 a	135.99 a	**137.34 A**
KL-46	123.43 b-e	121.01 d-g	**122.22 CD**
KL-29	127.25 b	124.76 bcd	**126.00 B**
KL-45	122.80 b-f	120.39 d-g	**121.59 CD**
KL-47	114.82 ı-n	112.57 k-p	**113.69 GH**
KL-58	110.97 l-r	108.80 p-v	**109.88 IJK**
KL-57	113.12 j-p	110.90 m-r	**112.01 HI**
KL-60	113.41 j-o	111.19 l-r	**112.30 HI**
KL-59	113.17 j-p	113.17 j-p	**114.30 GH**
KL-30	102.12 zA	102.12 zA	**101.12 O**
KL-44	92.93 B	91.11 B	**92.93 B**
FS-1	121.91 c-f	119.55 e-h	**120.74 DE**
FS-197	125.60 bc	123.14 b-e	**127.37 BC**
FS-141	120.73 d-g	118.37 f-ı	**119.55 DE**
FS-185	119.40 e-h	117.05 g-k	**118.22 EF**
FS-148	123.09 b-e	120.67 d-g	**121.88 CD**
FS-184	107.46 q-x	105.35 t-z	**106.40 LMN**
FC-44	112.51 l-p	110.31 n-s	**111.41 HI**
FC-73	111.47 l-q	109.28 o-t	**110.37 IJ**
FS-86	106.38 s-z	104.30 v-A	**105.34 LMN**
FC-76	106.16 s-z	104.08 w-A	**105.12 LMN**
KL-5	115.08 h-m	115.08 h-m	**116.23 FG**
FC-19	106.89 r-y	104.79 t-z	**105.84 LMN**
KL-6	86.56 D	84.86 CD	**85.71 Q**
KL-26	83.96 CD	82.32 DE	**83.14 QR**
FC-48	109.24 o-t	107.09 q-x	**108.16 JKL**
FS-43	105.11 t-z	103.05 x-A	**104.08 MNO**
FC-118	104.62 u-A	102.57 lzA	**103.59 NO**
FC-22	109.02 o-u	106.89 r-y	**107.95 JKL**
KL-1	85.69 D	84.01 CD	**84.85 Q**
KL-4	82.28 DE	80.67 D	**81.47 R**
FC-83	123.20 b-e	120.78 d-g	**121.99 CD**
PCPM4	104.33 v-A	102.28 zA	**103.30 NO**
GF-677	138.32 a	135.61 a	**136.96 A**
R-20	107.89 q-v	106.77 t-z	**106.83 KLM**
GN-22	139.84 a	137.10 a	**138.47 A**
Mean of Variety	**112.05 A**	**109.86 B**	
LSD	**Rootstock : 3.17****	**Variety: 0.74****	**RootstockxVariety : 4.49****

1-Different letters indicate that differences between means. In the interaction part, uppercase letters used after lowercase letters when comparing means. Rootstock means written in bold uppercase letters, variety means written in bold italic uppercase letters. 2- N.S.: Not Significant; **: p<0.01; *<0.05.3- Numbers in parentheses are arc transformation values.

DISCUSSION AND CONCLUSION

Rootstocks influence the growth vigor of the scion varieties grafted onto them (Gravite et al. 2018). This effect is crucial for determining planting density in orchard establishment (Yordanov et al. 2015). Depending on the growth vigor in fruit trees, the amount of fruit per unit stem cross-sectional area can vary (Miloseviç, 2011). Attention to these criteria is necessary in rootstock breeding studies. When examining the seedling development of peach varieties grafted onto selected and control rootstocks, significant differences were observed both between rootstocks and varieties. Particularly, combinations of rootstocks and scions with similar origins exhibited stronger growth, while rootstocks of P. domestica origin showed more dwarf growth. Additionally, various rootstock and scion combinations with different growth vigor were formed. It was determined that the differences in growth vigor among varieties stem from the effects of rootstocks on the varieties, and these differences are a significant result of the interaction between the rootstock and scion. It is thought that the primary reason for this situation is the different origins of the rootstocks. At the end of the study, it was observed that besides strong-growing rootstocks, dwarf rootstocks also exist. These rootstocks are understood to have the potential for use in high-density planting fruit orchards in the future.

ACKNOWLEDGEMENT

The results obtained from this article were taken from project number 119O266 supported by TÜBİTAK.

REFERENCES

Bolat,I.,Ak,B.E.,Acar,I.,Ikıncı,A.2017. Plum culture ın Turkey. Acta Horticultureae.1715:15-18. DOI 10.17660/ActaHortic.2017.1175.4

Chin SW, Shaw J, Haberle R, Wen J, Potter D (2014) Diversification of almonds, peaches, plums and cherries— molecular systematics and biogeographic history of Prunus (Rosaceae). Mol Phylogenet Evol 76:34–48. https://doi.org/10.1016/j.ympev.2014.02.024

FAO 2020. Food and Agriculture Organization. http://www.fao.org/faostat/en/#data/QC/visualize (Erişim Tarihi: 20.06.2020).

Gravite, I., Kaufmane, E., Cirsa, E., Lanauskas, J. 2018. Preliminary performance of six plum rootstocks on six European plum cultivars in Latvia. In *XXX International Horticultural Congress IHC2018: International Symposium on Cultivars, Rootstocks and Management Systems of 1281* (pp. 137-144).

Milosevic, T. 2011. Influence of stock on the early tree growth, yield and fruit quality traits of apricot (Prunus armeniaca L.). *Journal of Agricultural Sciences*, *17*(3).

Özdoğru B, Şen F, Bilgin N, Mısırlı A (2015). Bazı sofralık kayısı çeşitlerinin depolanma surecinde fiziksel ve biyokimyasal değişimlerinin belirlenmesi. Ege Üniversitesi Ziraat Fakültesi Dergisi 52: 23-30

Yordanov, I. A., Tabakov, G. S., Kaymakanov, V. P. 2015. Comparative study of Wavit® rootstock with two plum and two apricot cultivars in nursery. *Journal of Agricultural Sciences (Belgrade)*, *60*(2), 159-168.

INVESTIGATION OF GRAFT COMPATIBILITY RATES OF DIFFERENT CLONAL ROOTSTOCKS WITH SOME PEACH AND ALMOND VARIETIES

Remzi Uğur[1], Mehmet Ali Sarıdaş[2], Halit Seyfettin ATLI[3]*

[1]Gaziantep University Nurdagi Vocational School
[2]Cukurova University, Faculty of Agriculture, Department of Horticulture
[3]Siirt University Faculty of Agriculture Department of Horticulture

[*] Corresponding author, E-mail: remzibey@hotmail.com

ABSTRACT

The compatibility of selected rootstocks with fruit varieties is crucial in rootstock breeding programs. A high grafting success rate ensures the commercial acceptance of the rootstock. This study was conducted in the greenhouses of the Eastern Mediterranean Transitional Zone Agricultural Research Institute Directorate during the years 2020-2021. In the study, 33 selected rootstocks and 4 control rootstocks were used. The peach varieties used were Transvalia and Flored, while the almond varieties were Feragnes and Ferraduel. The grafting studies revealed significant differences (at the 1% level) in the grafting success rates of the rootstocks with the peach varieties. The highest grafting success rates were observed in GN-22 (91.50%), FC-19 (90.66%), Rootpack-20 (90.50%), FC-22 (89.50%), and GF-677 (88.66%). The average grafting success rate across all combinations was 73.33%. Out of the 38 rootstocks, 24 had a grafting success rate of 70% or higher, which was considered a positive result. The lowest grafting success rates were found in the rootstock candidates KL-30 (56.33%) and KL-44 (56.16%). In the almond varieties, the grafting success rates ranged from 57.66% to 96.00%. The highest grafting success rates were observed in GN-22 (96.00%), GF-677 (93.83%), and FC-19 (92.66%), while the lowest grafting success rates were found in KL-1 (57.66%), KL-6 (57.83%), and KL-4 (58.83%). The average grafting success rate for the almond varieties was 73.18%, with most rootstocks having a grafting success rate above 70%, which was considered a positive outcome. The study identified a significant number of clonal rootstocks that could be used for peach and almond fruit varieties.

Keywords: Rootstock candidates, graft compatibility, seedling development, peach rootstock, almond

INTRODUCTION

Stone fruits are among the most widely cultivated fruits globally, with peaches and almonds holding significant importance. Peach is one of the most extensively bred fruits. In recent years, a considerable number of new peach and nectarine varieties have been developed, leading to an increased interest among consumers and producers. Among cultivated fruit varieties, peach shows the greatest phenological differences (Reig et al. 2016). In the Northern Hemisphere, fresh peaches and nectarines can be found in markets for six months of the year. With advances in technology, greenhouse cultivation techniques have extended this calendar even further. These characteristics necessitate the cultivation of peaches in various environments and climates. Almond cultivation is an important agricultural sector worldwide, with significant economic, nutritional, health, environmental, and cultural value. In the Mediterranean region, almonds hold historical and cultural importance. The United States alone accounts for a large portion of global almond production, followed by Spain, Iran, and Italy. Almond cultivation is essential due to its economic value and rich nutritional content, which is beneficial for health. Almonds can be grown in a variety of soil and climate conditions. Given the diverse climate and soil conditions for peach and almond cultivation, the selection of rootstocks becomes crucial. This necessitates the acceleration of breeding programs to develop suitable rootstocks for these new varieties (Iglesias, Carbo, and Bonany 2009). This study was designed and conducted to determine the graft take rates of the rootstock-scion combinations using Transvalia and Flored peach varieties. The study was conducted in the greenhouses and fields of the Eastern Mediterranean Transitional Zone Agricultural Research Institute Directorate in 2019-2020.

MATERIAL AND METHOD

The materials for the study consisted of 33 clonal rootstocks obtained through selection and hybrid breeding of the genus *Prunus* (Table 1).

Table-1. Rootstock candidates used in grafting study

No	**Origin of Rootstocks**	**Clon No**
1	*P.cerasifera*	KL-29
2	*P.cerasifera*	KL-45
3	*P.cerasifera*	KL-59
4	*P.cerasifera*	KL-38
5	*P.divaricata*	KL-46
6	*P.cerasifera*	KL-57
7	*P.divaricata*	KL-47
8	*P.cerasifera*	KL-58
9	*P.domestica*	KL-6
10	*P.cerasifera*	KL-60
11	*P.domestica*	KL-1
12	*P. amygdalus x P. cerasifera*	FC118
13	*P. amygdalus x P. cerasifera*	FS-148
14	*P. amygdalus x P. cerasifera*	FS-185
15	*P.divaricata*	KL-5
16	*P.domestica*	KL-30
17	*P.domestica*	KL-44
18	*P. amygdalus x P. cerasifera*	FS-197
19	*P. amygdalus x P. cerasifera*	FS-1
20	*P.domestica*	KL-26
21	*P. amygdalus x P. cerasifera*	FS-141
22	*P. amygdalus x P. cerasifera*	FC22
23	*P. amygdalus x P. cerasifera*	FC83
24	*P.divaricata*	KL-4
25	*P. amygdalus x P. cerasifera*	FC19
26	*P. amygdalus x P. cerasifera*	FC44
27	*P. amygdalus x P. cerasifera*	FC48
28	*P. amygdalus x P. cerasifera*	FC73
29	*P. amygdalus x P. cerasifera*	FC76
30	*P. amygdalus x P. cerasifera*	FS-43
31	*P. amygdalus x P. cerasifera*	FS-86
32	*P. amygdalus x P. cerasifera*	FS-184
33	*P. microcarpa x P. cerasifera*	PCXPM4

Transvalia and Flored peach varieties were grafted onto selected and control rootstocks within the scope of the trial. The graft take rates of the seedlings were recorded two months after grafting. Statistical analysis was applied to the obtained values, thus revealing the compatibility of rootstocks with the varieties. The study was established in a randomized block design with three replications, each replication consisting of five plants. Variance analysis was applied to the obtained data ($P<0.05$). Comparison of means was performed using the LSD test.

RESULTS

The graft take rates of Flored and Transvalia peach varieties on selected and control rootstocks are given in Table 2. The differences in graft take rates between rootstocks, varieties, and rootstock/variety interactions were found to be statistically significant. The graft take rate of Transvalia variety was found to be 74.89%, higher than that of Flored variety (71.79%). It was determined that the rootstocks grafted with peach varieties showed statistically significant differences in graft take rates at the 1% level, with the highest graft take rates observed in GN-22 (91.50%), FC-19 (90.66%), Rootpack-20 (90.50%), FC-22 (89.50%), and GF-677 (88.66%) rootstocks among the selected and control rootstocks. In the distribution where the average graft take rate was 73.33%, it was considered positive that 24 out of 38 rootstocks had a graft take rate of 70% and above. The lowest graft take rates were found in rootstock candidates KL-30 (56.33%) and KL-44 (56.16%). It was determined that the graft take rates also showed statistically significant differences at the 1% level in rootstock/scion interactions, with these rates ranging from 54.66% to 96.33%. The highest graft take rates were calculated in GN-22/Transvalia (96.33%), GF-677/Transvalia (94.33%), FC-19/Transvalia (93.33%), and Rootpack-20/Transvalia (91.00%) combinations. It was noted that the Transvalia variety formed compatible combinations with selected and control rootstocks, and that Rootpac-20 and FC-22 rootstocks achieved high graft take rates with both peach varieties. In general, plum group rootstocks did not achieve very high graft take rates with peach. It was determined that hybrid rootstocks had higher graft take rates overall, while the KL-38/Transvalia combination had a remarkably high graft take rate of 82.00%. The lowest graft take rates were found in KL-30/Transvalia (54.66%), KL-44/Transvalia (56.00%), KL-44/Flored (56.33%), KL-30/Transvalia (58.00%), KL-1/Flored (60.00%), and KL-4/Flored (60.66%) combinations. In general, it was observed that graft take rates of 70% and above were achieved in rootstock/scion interactions.

The graft take rates of Ferragnes and Ferraduel almond varieties grafted onto selected and control rootstocks were found to be similar, with no statistically significant difference between them. The graft take rate averages of the varieties were very close, calculated as 73.26% for Ferragnes and 73.11% for Ferraduel (Table 3).

Table 2. Graft take rates of peach varieties grafted onto rootstocks (%)

Rootstocks	**Peach Varieties**		**Mean of Rootstocks**
	Flored	**Transvalia**	
KL-29	67.33 (55.14)[3] x-I[1]	67.33 (55.14) x-I	**67.33 (55.14) M-P**
KL-45	66.66 (54.75) z-J	64.66 (53.54) D-K	**65.66 (54.14) OPQ**
KL-59	70.00 (57.79) v -F	72.66 (58.48) q-A	**71.33 (57.63) G-M**
KL-38	71.00 (57.42) t-D	82.00 (64.94) h-k	**76.50 (61.18) DEF**
KL-46	64.00 (53.13) E-K	71.66 (57.91) s-C	**67.83 (55.52) L-P**
KL-57	66.00 (54.34) A-J	71.66(57.86) s-D	**68.83 (56.10) K-O**
KL-47	67.00 (54.94) y-J	70.00 (58.87) u-E	**68.50 (55.90) L-O**
KL-58	68.00 (55.55) w-H	73.00 (58.69) p-z	**70.50 (57.12) I-O**
KL-6	68.00 (55.56) w-H	66.33 (54.54) z-J	**67.16 (55.05) M-P**
KL-60	65.00 (53.76) B-K	75.33 (60.24) n-v	**70.16 (57.00) J-O**
KL-1	60.00 (50.78) J-M	62.33 (52.16) G-L	**61.16 (51.47) QR**
FC118	68.00 (55.57) w-H	80.00 (64.62) h-m	**74.00 (60.09) D-I**
FS-148	67.33 (55.15) x-I	77.00 (61.60) k-t	**72.16 (58.38) F-L**
FS-185	71.66 (57.85) s-D	73.66 (59.13) p-y	**72.66 (58.49) F-L**
KL-5	67.33 (55.14) x-I	65.00 (55.73) C-K	**66.16 (54.44) N-Q**
KL-30	58.00 (49.61) J-K	54.66 (47.67) M	**56.33 (48.64) R**
KL-44	56.33 (48.64) L-M	56.00 (48.47) L-M	**56.16 (48.56) R**
FS-197	67.33 (55.16) x-I	77.00 (59.34) o-x	**70.66 (57.25) H-N**
FS-1	71.66 (57.86) s-D	79.33 (62.97) j-p	**75.50 (60.41) D-G**
KL-26	64.00 (53.13) E-K	63.00 (52.53) F-L	**63.50 (52.83) PQ**
FS-141	72.66 (58.50) q-A	78.00 (62.03) k-s	**75.33 (60.26) D-H**
FC22	90.00 (71,67) cde	89.00 (70.64) def	**89.50 (71.15) A**
FC83	83.66 (66.39) f-j	80.00 (63.46) j-o	**81.83 (64.93) BC**
KL-4	60.66 (51.16) I-M	62.00 (51.94) H-M	**63.33 (51.55) QR**
FC19	88.00 (69.74) d-g	93.33 (75.22) abc	**90.66 (72.48) A**
FC44	84.00 (66.42) f-j	86.00 (68.03) e-ı	**85.00 (67.23) B**
FC48	77.33 (61.57) k-t	78.66 (62.51) j-q	**78.00 (62.04) CDE**
FC73	74.33 (58.62) o-w	78.66 (62.53) j-q	**76.50 (61.07) DEF**
FC76	75.33 (60.31) m-v	78.33 (62.28) j-r	**76.83 (61.29) DEF**
FS-43	69.33 (56.39) v-G	77.33 (61.67) k-t	**73.33 (59.03) E-K**
FS-86	81.00 (64.19) ı-n	76.66 (61.14) l-u	**78.83 (62.67) CB**
FS-184	72.66 (58.52) q-A	76.66 (61.14) l-u	**74.66 (59.83) D-J**
PCXPM4	75.66 (60.60) m-v	77.00 (61.36) l-t	**76.33 (60.98) DEF**
MYROBOLAN	67.33 (55.17) x-I	65.66 (54.13) B-J	**66.50 (54.65) M-P**
GF-677	83.00 (65.83) g-k	94.33 (76.47) ab	**88.66 (71.15) A**
GN-22	86.66 (68.74) d-h	96.33 (79.51) a	**91.50 (74.12) A**
ROOTPAC-20	90.00 (71.67) cde	91.00 (72.55) bcd	**90.50 (72.11) A**
PİXY	72.00 (58.08) r-B	67.33 (55.18) x-I	**69.66 (56.63) K-O**
Mean varieties	**71.79 (60.59) A**	**74.89 (58.29) B**	**Rootstock mean:73.33**
[2]LSD0.05	**Rootstock: 3.04** **Rootstock*Variety: 4.30**		**Variety: 0.68**

1-Different letters indicate that differences between means. In the interaction part, uppercase letters used after lowercase letters when comparing means. Rootstock means written in bold uppercase letters, variety means written in bold italic uppercase letters. 2- N.S.: Not Significant; **: p<0.01; *<0.05.3- Numbers in parentheses are arc transformation values.

Table 3. Graft take rates of almond varieties grafted onto rootstocks (%)

Rootstocks	Almond Varieties		Mean of Rootstocks
	Ferragnes	Ferraduel	
KL-29	65.66 (54.13)[3] v-B[1]	64.66 (53.73) y-B	**65.16 (53.83) MN**
KL-45	64.66 (53.53) y-B	65.33 (53.93) w-B	**65.00 (53.73) MN**
KL-59	66.00 (54.33) v-B	65.00 (53.73) x-B	**65.50 (54.03) MN**
KL-38	75.00 (60.05) k-s	75.00 (60.01) k-s	**75.00 (60.03) H-K**
KL-46	66.00 (54.33) v-A	66.66 (54.74) u-A	**66.33 (54.53) MN**
KL-57	66.33 (54.54) v-A	65.00 (53.73) x-B	**65.66 (54.14) MN**
KL-47	66.33 (54.54) v-A	70.00 (56.79) s-y	**68.16 (55.67) LM**
KL-58	64.33 (53.36) y-B	64.00 (53.15) Z-D	**64.16 (53.25) MNO**
KL-6	57.66 (49.60) D-G	58.00 (49.60) C-G	**57.83 (49.51) Q**
KL-60	64.00 (53.13) z-E	66.00 (54.33) v-B	**65.00 (53.73) MN**
KL-1	74.00 (50.98) B-G	55.00 (47.87) FG	**57.66 (49.42) Q**
FC118	74.00 (59.37) L-S	73.33 (58.92) n-s	**73.66 (59.15) JK**
FS-148	73.66 (59.15) l-s	77.00 (61.34) j-p	**75.33 (60.24) G-K**
FS-185	72.33 (58.26) p-u	71.00 (57.63) q-v	**71.83 (57.95) KL**
KL-5	73.00 (58.71) o-t	71.00 (57.42) r-w	**72.00 (58.06) KL**
KL-30	62.66 (52.34) z-E	58.33 (49.80) C-G	**60.50 (51.07) OPQ**
KL-44	86.00 (53.19) z-D	54.33 (47.87) a	**59.16 (50.34) PQ**
FS-197	72.66 (58.48) o-t	70.66 (57.21) r-x	**71.66 (57.84) KL**
FS-1	72.33 (58.36) p-t	75.66 (60.45) k-r	**74.00 (59.40) IJK**
KL-26	70.66 (57.42) r-w	72.66 (58.52) o-t	**71.83 (57.97) KL**
FS-141	78.00 (62.03) ı-o	77.00 (61.40) j-p	**77.50 (61.71) F-I**
FC22	79.00 (62.73) ı-l	78.66 (62.50) l-m	**78.83 (62.61) EFG**
FC83	80.00 (63.46) ıjk	70.00 (56.79) s-y	**75.00 (60.13) G-K**
KL-4	60.33 (50.98) B-a	57.33 (49.22) FG	**58.83 (50.09) PQ**
FC19	93.33 (75.22) bc	92.00 (73.70) cd	**92.66 (74.46) B**
FC44	86.00 (68.03) fgh	91.66 (73.31) cd	**88.83 (70.67) C**
FC48	78.66 (62.51) ı-m	84.66 (67.10) gh	**81.66 (64.81) DE**
FC73	78.66 (62.53) ı-m	82.33 (65.29) hı	**80.50 (63.91) DEF**
FC76	78.33 (62.28) ı-n	81.33 (64.61) ıjk	**79.83 (63.44) DEF**
FS-43	77.33 (61.67) J-P	87.33 (69.74) efg	**82.33 d(65.70) D**
FS-86	76.66 (61.14) j-q	78.66 (62.50) ı-m	**77.66 (61.82) F-I**
FS-184	76.66 (61.14) j-q	79.00 (62.74) ı-l	**77.83 (61.94) FGH**
PCXPM4	77.00 (61.36) j-p	77.33 (61.57) j-p	**77.16 (61.47) F-J**
MYROBOLAN	67.66 (55.35) t-z	61.00 (51.35) A-F	**64.33 (53.35) MNO**
GF-677	94.33 (76.47) abc	93.33 (75.39) bc	**93.83 (75.93) B**
GN-22	96.33 (79.51) a	95.66 (78.06) ab	**96.00 (78.78) A**
ROOTPAC-20	91.33 (72.92) cde	89.33 (70.96) def	**90.33 (71.94) C**
PİXY	62.66 (52.14) z-E	62.66 (52.34) Z-E	**62.50 (52.24) NOP**
Mean varieties	***73.26 (59.45)***	***73.11 (59.44)***	**Rootstock mean:73.18**
[2]LSD0.05	**Rootstock : 2.50****	**Variety: N.S.**	**Rootstock*Variety : 3.54****

1-Different letters indicate that differences between means. In the interaction part, uppercase letters used after lowercase letters when comparing means. Rootstock means written in bold uppercase letters, variety means written in bold italic uppercase letters. 2- N.S.: Not Significant; **: p<0.01; *<0.05.3- Numbers in parentheses are arc transformation values.

The differences in graft take rates between selected and control rootstocks were found to be statistically significant at the 1% level. The graft take rates of the rootstocks ranged from 57.66% to 96.00%. The highest graft take rates were found in GN-22 (96.00%), GF-677 (93.83%), and FC-19 (92.66%) rootstocks, while the lowest graft take rates were found in KL-1 (57.66%), KL-6 (57.83%), and KL-4 (58.83%) rootstocks. In general, hybrid rootstocks showed higher graft take rates with almond varieties, possibly due to genetic proximity. In the distribution with an average graft take rate of 73.18%, it was considered positive that most rootstocks had graft take rates above 70%. It was observed from Table 29 that the combinations of both almond varieties with selected and control rootstocks showed statistically significant differences in graft take rates at the 1% level. In this distribution, graft take rates ranged from 54.33% to 96.33%, with the highest rates observed in seven combinations within the same statistical group. The highest values were found in GN-22/Ferragnes (96.33%), GN-22/Ferraduel (95.66%), and GF-677/Ferraduel (94.33%) combinations, while the lowest rates were observed in KL-44/Ferraduel (54.33%) and KL-1/Ferraduel (55.00%) combinations. In general, it was observed from Table 2 that plum group rootstocks showed lower graft take rates with almond varieties, while hybrid rootstocks achieved higher rates.

A-Aşılama için yonga açılması
B-Anaç gövdesinde açılmış yonga
C-Aşılama işlemi tamamlanıp bağlanmış göz.
D-Aşılamanın ardından sürmüş bir göz.
E-Aşılamayı müteakip erken dönem aşı uyuşması yaşanmadığı düşünülen ve çalışmaya dâhil edilen fidan
F-Ölçüm işlemleri devam etmekte olan ve sağlıklı gelişen bir fidan

Figure 7. Observations on grafting and growing grafted buds.

Overall, the results of the grafting trials on the examined rootstocks and scions showed that graft take rates averaged above 54.66%, with the highest average reaching up to 96.00%. The study found that standard rootstocks generally had high graft take rates, with some selected rootstocks showing similarly high rates. The presence of many selected rootstock candidates with graft take rates between 75.00% and 94.66% suggests that the research conducted successfully and met its objectives. These results are promising for providing solutions to the rootstock needs of some temperate climate fruit species in Turkish horticulture. Approximately 1.5 months after grafting, the growth and development of the shoots measured and observed as shown in Figure-7.

DISCUSSION AND CONCLUSSION

Rootstocks affect the vigor of the varieties grafted onto them (Gravite et al., 2018). This effect is quite important in determining planting density in orchards (Yordanov et al., 2015). The amount of fruit per unit trunk cross-sectional area can vary depending on the vigor of the fruit trees (Milosevic, 2011). These criteria should be considered in rootstock breeding studies. When examining the growth status of peach varieties grafted onto selected and control rootstocks, significant differences observed between rootstocks and varieties. Stronger growth observed in combinations with similar origins, while more dwarf growth observed in rootstocks derived from *P. domestica*. Additionally, various combinations of rootstocks and scions exhibited different growth vigor. The overall results of the grafting trials on the examined rootstocks and scions showed that graft take rates averaged above 54.66%, with the highest average reaching up to 96.00%. The study found that standard rootstocks generally had high graft take rates, with some selected rootstocks showing similarly high rates. The presence of many selected rootstock candidates with graft take rates between 75.00% and 94.66% suggests that the research conducted successfully and met its objectives. These results are promising for providing solutions to the rootstock needs of some temperate climate fruit species in Turkish horticulture. Approximately 1.5 months after grafting, the growth and development of the shoots were measured and observed as shown in Figure-7. It has been determined that the differences in growth vigor are due to the effects of the rootstock on the variety and that these differences are a significant result of the interaction between the rootstock and the scion. This situation is thought to be primarily due to the different origins of the rootstocks. At the end of the study, it was observed that, in addition to rootstocks showing strong growth, there are also dwarf rootstocks. It was understood that these rootstocks might have the potential to be used in high-density fruit orchards in the future.

ACKNOWLEDGEMENT

The results obtained from this article were taken from project number 119O266 supported by TÜBİTAK.

REFERENCES

Bolat,I.,Ak,B.E.,Acar,I.,Ikıncı,A.2017. Plum culture ın Turkey. Acta Horticultureae.1715:15-18. DOI 10.17660/ActaHortic.2017.1175.4

Chin SW, Shaw J, Haberle R, Wen J, Potter D (2014) Diversification of almonds, peaches, plums and cherries— molecular systematics and biogeographic history of Prunus (Rosaceae). Mol Phylogenet Evol 76:34–48. https://doi.org/10.1016/j.ympev.2014.02.024

FAO 2020. Food and Agriculture Organization. http://www.fao.org/faostat/en/#data/QC/visualize (Erişim Tarihi: 20.06.2020).

Gravite, I., Kaufmane, E., Cirsa, E., Lanauskas, J. 2018. Preliminary performance of six plum rootstocks on six European plum cultivars in Latvia. In *XXX International Horticultural Congress IHC2018: International Symposium on Cultivars, Rootstocks and Management Systems of 1281* (pp. 137-144).

Milosevic, T. 2011. Influence of stock on the early tree growth, yield and fruit quality traits of apricot (Prunus armeniaca L.). *Journal of Agricultural Sciences*, *17*(3).

Özdoğru B, Şen F, Bilgin N, Mısırlı A (2015). Bazı sofralık kayısı çeşitlerinin depolanma surecinde fiziksel ve biyokimyasal değişimlerinin belirlenmesi. Ege Üniversitesi Ziraat Fakültesi Dergisi 52: 23-30 (In Turkish)

Yordanov, I. A., Tabakov, G. S., Kaymakanov, V. P. 2015. Comparative study of Wavit® rootstock with two plum and two apricot cultivars in nursery. *Journal of Agricultural Sciences (Belgrade)*, *60*(2), 159-168.

USAGE OF PLANT ESSENTIAL OILS IN THE CONTROL OF PATULIN PRODUCING *Penicillium* SPECIES

İlayda ÇETİN[1], *Filiz ÜNAL*[1*]

[1]*Department of Plant Protection, Faculty of Agriculture, Osmangazi University, 26160 Eskisehir, Türkiye*
Corresponding author e-mail: filiz.unal@ogu.edu.tr

ABSTRACT

Patulin is a toxin produced by some toxigenic species of fungi belonging to the *Penicillium*, *Aspergillus* and *Byssochlamys* genera. It is a type of enteropathogenic mycotoxin, it is rapidly absorbed by the intestines and can have toxic, mutagenic and cytotoxic effects in many living things. Patulin can be found especially in apples and apple products, and rarely in peaches, grapes, tomatoes, oranges and products derived from them. The most common patulin-producing fungus is *Penicillium expansum*. The main method to prevent and control the spread of patulin in food products is to prevent the development and production of patulin-producing fungi before and after harvest. Chemical and physical methods used to prevent patulin formation in apple fruits and derivatives, where patulin formation is most commonly observed, are expensive and may negatively affect product quality. Chemical applications require a thorough understanding of whether reaction products are toxic. In addition, some of these chemicals can seriously reduce the quality of the product by destroying its nutritional content and taste. Today, biological methods, such as the direct degradation of patulin by an antagonist microorganism or more natural factors such as plant extracts, stand out as the healthiest practices today. When the studies on the effects of some plant extracts on *Penicillium expansum*, the most important patulin producer, and patulin production are examined, *Ocimum basilicum* (basil), *Foeniculum sativum* (fennel), *Lavandula officinalis* (lavender), *Origanum majorana* (bridegroom), *Oreganum vulgare* (thyme), *Satureja montana* (winterwort), *Mentha arvensis* (mint), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Thymus vulgaris* (thyme) essential oils were investigated. Studies have yielded promising results for plant essential oils.

Key words: Essential oil, *Penicillium*, patulin, apple

INTRODUCTION

Mycotoxins are natural toxins with low molecular weight and a wide variety of chemical structures, formed as a result of the secondary metabolism of fungi such as *Aspergillus, Penicillium, Fusarium, Alternaria* and *Claviceps*. They have strong and diverse toxic effects on human and animal health (Steyn and Stander, 1999). Patulin (PAT) is a toxic metabolite produced by various *Penicillium, Aspergillus* and *Byssochlamys* species (Harrison, 1989).

It usually contaminates cereals, vegetables, fruits and their derivatives, especially apples and derivatives. Patulin can easily enter and accumulate in the processing of final products due to its water-soluble and heat-resistant properties (Moake et al., 2005). Patulin also contaminates many other foods such as apples, dried figs and corn. Currently, patulin is considered a global toxin and many countries monitor its residues in foods (Zhao et al., 2018). Patulin can be seen in highly acidic fruits, vegetables and products such as apples, apple juice and concentrate, apple jam and candied fruit, pears, apricots, oranges, peaches, and tomatoes (Tunail, 2000). Among mycotoxins, patulin (4-hydroxy-4H-furo[3,2-c] pyran-2(6H)-one) is a polyketide lactone with a molar mass of 154.12 g/mol and a melting point of 110℃. It is soluble in water, stable under acidic conditions and heat, and cannot be thermally denatured (Saleh and Göktepe 2019a; Zheng et al., 2020).

Patulin was first isolated from *Penicillium griseofulvumin* by Harold Raistrick in 1943 (Saleh and Göktepe, 2019a). Shortly after its identification, patulin was investigated as an antimicrobial agent against some gram-positive and gram-negative bacteria under the name of "tercinin" at the British Medical Research Center. However, it did not take long for researchers at the center to detect its toxic effects in 1944 (Saleh and Göktepe, 2019b). Its activity as an antimicrobial was seen to be very promising, as it proved to be up to ten times more effective in the treatment of infections caused by *Bacillus* ssp. At that time, the substance was selected from *Penicillium patulum* (later named *Penicillium urticae* and today known as *Penicillium griseofulvum*). However, the emergence of its toxic effects in both plants and animals between 1950 and 1960 prevented its clinical use as an antibiotic. Since the 1960s, patulin has been reclassified and is now known as a toxic secondary metabolite of fungal origin, i.e. a mycotoxin (Basso, 2019). To determine the maximum patulin production by different Penicillium species, Dombrink-Kurtzman and Blackburn (2005) compared six different culture media. 10 strains of *Penicillium expansum, Penicillium griseofulvum, Penicillium clavigerum* and *Penicillium coprobium*, which were previously identified as patulin producers, were studied. Three types of liquid media (PDB, MEB and 5-GYEP) were tested in manganese-free and 152 μmol manganese-containing forms (6 media). The flasks were incubated in a shaking incubator (240 rpm) at 25°C for 96 hours, then filtered, extracted and analyzed by HPLC. After 96 hours, P. griseofulvum isolates were determined to be the highest patulin producers (Table 1).

Table 1. Patulin-Producing Fungi

Patulin-Producing Fungi	*Penicillium expansum*	Hohler, 1998
	Penicillium patulum	
	Aspergillus terreus	
	Aspergillus giganteus	
	Aspergillus clavatus	
	Penicillium aurantiogriseum (Penicillium cyclopium)	
	Penicillium equinum (Penicillium Terrestre)	
	Penicillium melinii	
	Penicillium claviforme	
	Byssochlamys nivea	
	Byssochlamys fulva (Paelomyces variotii)	
	Penicillium roquefortii	

Thirteen *Penicillium* species can produce patulin, including *P. expansum*, a major patulin-producing species (Bokhari et al., 2009).

According to the World Health Organization, the maximum acceptable patulin level in apple juice has been determined as 50 µg/L (Saleh and Göktepe, 2019a). This value is in line with the recommendations of the Food and Drug Administration (FDA), the National Health Surveillance Agency (ANVISA) and the European Union (EU), the latter of which has limited the patulin level in solid apples to 50 µg (Basso, 2019). According to the recommendation of the European Commission and based on the determined patulin level (43 µg/kg body weight), the maximum daily patulin dose allowed temporarily has been fixed at 0.4 µg/kg body weight. This level has been adopted in most health risk assessment analyses conducted on patulin (Saleh and Göktepe, 2019a).

Due to the high prevalence of patulin, concerns have arisen regarding the toxicological effects of patulin on humans and animals through consumption. Patulin causes various chronic health effects on the genetic, immune and central nervous systems in animals, although its effects on humans are not yet clear (Sajid et al., 2018).

CONTROLING PATULIN

Physical methods include separation, high-pressure water washing of fruits, cooling, juice clarification, filtration, adsorption, pasteurization, and radiation for processing raw materials such as apples to prevent patulin contamination. Physical methods provide good results but require significant labor and material resources; they are expensive and may even affect product quality (Zheng et al., 2020). Chemical methods include ammonia and potassium permanganate, which can reduce patulin content in fruit juice by more than 99%. This technique is the most effective way to detoxify patulin, but it causes serious damage to product quality. In addition, sulfur dioxide, ascorbic acid, N-acetylcysteine, and glutathione and calcium D pantothenate can reduce the amount of patulin in fruit juice. However, the addition of these chemicals requires a thorough understanding of the reaction mechanism, that is, whether the reaction products are toxic or not. In addition, some of these chemicals can seriously reduce the quality of the product by destroying its nutritional content and flavor (Zheng et al., 2020). Biological methods, especially the direct degradation of patulin by an antagonist microorganism, have shown great promise for application in the control of patulin contamination. However, several issues need to be considered before using these microorganisms. First of all, it is necessary to investigate whether patulin is adsorbed or degraded by microorganisms. Degradation of patulin by microorganisms can prevent further overprocessing of patulin if it is converted to a less toxic or non-toxic compound. For this, patulin can be reduced through an enzymatic effect (Zheng et al., 2020).

Although physical-chemical methods have varying degrees of success, limited effectiveness and loss of important nutrients still prevent their application in food industries. Biological methods are considered a promising solution for mycotoxin decontamination and are widely investigated (Li et al., 2019).

The demand for alternative methods is rapidly increasing as consumers are less likely to prefer fruits preserved with synthetic fungicides against infections. Plant extracts are one of these alternative methods. Many plants have natural antimicrobial potential as they contain phytochemicals that exhibit antimicrobial and cytotoxic effects on microorganisms. Since they are non-toxic and environmentally and nature-friendly, they have potential as an alternative to synthetic fungicides in application to agricultural products and foods (Salhi et al., 2017; El-shahir et al., 2022).

The use of naturally obtained chemicals from plants that prevent the reproduction and development of plant pathogenic fungi will be a more realistic and ecologically reliable method in the integrated control of plant diseases and will play an important role in the development of commercial pesticides for plant protection strategies in the future (Yanar et al., 2011, Hubert et al., 2013).

Volatile oils obtained from plant materials by various extraction methods have been investigated for years in our country and in the world for their effects against plant pathogens. Today, there are many plant by-products that have antimicrobial properties against many plant pathogenic bacteria and fungi and that occur together with the main substance obtained from plants (Satish et al. 1999). The basic method to prevent and control the spread of patulin, an important mycotoxin, in apples and its products is to prevent the growth and patulin production of patulin-producing strains during the pre-harvest and post-harvest periods of apples (Moake et al., 2005).

ANTIFUNGAL EFFECTS OF ESSENTIAL OILS

In a study conducted on apple fruits, the antifungal effects of the essential oils of *Ocimum basilicum* (basil), *Foeniculum sativum* (fennel), *Lavandula officinalis* (lavender), *Origanum majorana* (oregano), *Origanum vulgare* (thyme), *Satureja montana* (zathane), *Mentha arvensis* (mint), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), and *Thymus vulgaris* (thyme) were investigated against *Botrytis cinerea* and *Penicillium expansum*. As a result of the study, it was determined that *O. vulgare, S. montana* and *T. vulgaris* showed high antifungal effects in 1% emulsions. In 10% emulsions of essential oils, it caused phytotoxicity in the fruit (Lopez-Reyes et al., 2010). In studies conducted on essential oils obtained from thyme and thyme oil, the inhibitory effect of 10-500 µl/L carvacrol and thymol was tested in petri dishes containing PDA. As a result of the studies, thymol (100 µl/L) inhibited the growth of *Penicllium italicum* and *P. digitatum* by 68% and 47%, respectively (Pérez-Alfonso et al., 2012)

The antifungal effect of essential oils of 25 different plants against *Penicllium expansum, P. brevicompactum, Fusarium oxysporum, F. verticillioides, Aspergillus flavus, A. fumigatus* agents was investigated, and it was determined that *Pimenta dioica* essential oil showed the highest effect among these plants (Zabka et al., 2009)

In a study investigating the antifungal effects of essential oil components of plants growing wild in Greece such as *Salvia pomifera* subsp. *calycina, Salvia fruticosa, Satureja thymbra ve Origanum onites* against *Aspergillus niger, A. flavus, P. expansum, Phomopsis helianthi, Trichoderma viride, Cladosporium cladosporioides, Alternaria alternata*, it was determined that the essential oil with the lowest antifungal activity was sage, while the highest and broadest activity was found in the essential oils of *O. onites* and *S. thymbra* containing carvacrol. Among the tested components, it was determined that carvacrol had the highest antifungal effect and 1,8-cineole had the lowest effect (Sokovic et al., 2002).

In a study, lemon and orange essential oils were tested and it was found that patulin production by P. expansum in apples was completely inhibited with 0.2% lemon oil solution and more than 90% with 0.05% lemon oil and 0.2% orange oil solutions. Nguefack et al. (2012) reported that in addition to Ocimum gratissimum essential oil, the combination of Cymbopogon citratus, Ocimum gratissimum and Thymus vulgaris was also effective in reducing patulin levels. Other effective essential oils were tea tree, orange and lemon essential oils. In another study, it was found that the use of essential oils such as clove and cinnamon oil was effective in reducing patulin levels in apples (Sivakumar and Bautista-Baños, 2014). Similarly, it has been reported that enzymes (such as polyphenol oxidase and peroxidase) formed as a result of the use of garlic extract reduce patulin levels in fruits (Chen et al., 2023). For example, polyphenol oxidase extracted from apples has been shown to effectively reduce patulin levels in apple juice.There are many other studies on the use of essential oils against toxin producing fungi. Some of these studies are given in Table 2.

Table 2. Essential oils used against patulin-producing fungi

Pathogen	**Plants whose essential oils are used**	**References**
Penicillium digitatum *P. italicum* *Geotrichum citri-aurantii*	*Thymus leptobotrys* *Thymus satureioides* *Thymus broussonnetii* *Thymus riatarum*	Boubaker et al., 2016
Aspergillus flavus	*Origanum majorana*	Chaudhari et al., 2020a
Aspergillus flavus	*Ocimum sanctum* *O. basilicum* *O. canum*	Kumar A. et al., 2020
Penicillium digitatum *P. italicum*	*Lavandula angustifolia* *Origanum vulgare* *O. majorana* *Thymus vulgaris*	Plaza et al., 2004
Penicillium expansum	*Thymus vulgaris* *Origanum vulgare* *Lavandula angustifolia* *Mentha pipereta* *Eucalyptus globules*	Fincheira et al., 2023
Penicillium chrysogenum, *Penicillium digitatum,* *Penicillium expansum,* *Penicillium italicum,* *Penicillium sp.*	*Satureja cilicica* *Satureja cuneifolia* *Satureja hortensis* *Satureja montana* *Satureja spicigera* *Satureja thymbra*	Kordali et al., 2022
Penicillium expansum	*Origanum minutiflorum* *Mentha pulegium* *Teucrium polium*	Taş, 2015
Penicillium expansum *Rhizopus stolonifer* *Aspergillus niger* *Botrytis cinerea* *Colletotrichum gloeosporioides* *Alternaria mali* *Monilinia fructigena*	*Salvia rosmarinus* *Salvia officinalis* *Foeniculum vulgare* *Eucalyptus spp.* *Thymus spp.*	Yılmaz, 2012

CONCLUSIONS

Natural toxins formed as a result of secondary metabolism of fungi such as *Penicillium, Aspergillus Fusarium, Alternaria* and *Claviceps* are called mycotoxins. Patulin produced by *Penicillium, Aspergillus* and *Byssochlamys* species poses a danger to human and animal health. Although physical methods are successful in combating, there are difficulties due to limited effectiveness and loss of nutrients. Biological methods are accepted as a promising solution in the detoxification of mycotoxins and are being researched. Some chemicals used in chemical combat are not preferred because they cause degradation of nutrients and damage to flavor. As a result, the demand for alternative methods in combat methods is rapidly increasing. Essential oils obtained from plant materials by various methods are used as a combat method and their effects are being investigated. It has been determined that essential oils containing chemicals such as thymol and carvacrol in their components have high antifungal activity in many studies. It is cheaper, applicable and does not threaten human and environmental health compared to other methods. More research is needed in this sense.

REFERENCES

Basso, T. (2019). Quantificação de Patulina em sucos de maçã disponíveis no Mercado Sul Brasileiro (Doctoral dissertation).

Bokhari, F., Gherbawy, Y., and Najjar, A. (2009). Detection of the patulin-producing potential of some Paecilomyces variotii strains isolated from the air samples of Jeddah City, Saudi Arabia, using the RAPD-PCR technique. *Aerobiologia*, *25*, 49-54.

Boubaker, H., Karim, H., El Hamdaoui, A., Msanda, F., Leach, D., Bombarda, I., et al. (2016). Chemical characterization and antifungal activities of four Thymus species essential oils against postharvest fungal pathogens of citrus. Indus. Crops Prod. 86, 95–101. doi: 10.1016/j.indcrop.2016.03.036.

Chaudhari, A. K., Singh, V. K., Das, S., Prasad, J., Dwivedy, A. K., et al. (2020a). Improvement of in vitro and in situ antifungal, AFB1 inhibitory and antioxidant activity of Origanum majorana L. essential oil through nanoemulsion and recommending as novel food preservative. Food Chem. Toxicol. 143:111536. doi: 10.1016/j.fct.2020.111536.

Chen, Y., Xing, M., Chen, T., Tian, S., and Li, B. (2023). Effects and mechanisms of plant bioactive compounds in preventing fungal spoilage and mycotoxin contamination in postharvest fruits: a review. Food Chem. 415, 135787.

Dombrink-Kurtzman, M. A., and Blackburn, J. A. (2005). Evaluation of several culture media for production of patulin by Penicillium species. *International Journal of Food Microbiology*, *98*(3), 241-248.

El-Shahir, A. A., El-Wakil, D. A., Abdel Latef, A. A. H., and Youssef, N. H. (2022). Bioactive Compounds and Antifungal Activity of Leaves and Fruits Methanolic Extracts of Ziziphus spina-christi L. Plants, 11(6), 746.

Fincheira, P., Jofré, I., Espinoza, J., Levío-Raimán, M., Tortella, G., Oliveira, H. C., ... and Rubilar, O. (2023). The efficient activity of plant essential oils for inhibiting Botrytis cinerea and Penicillium expansum: Mechanistic insights into antifungal activity. *Microbiological Research*, *277*, 127486.

Harrison, M.A., (1989). Presence and stability of patulin in apple products: A review Journal of Food Safety, 9 (1989), pp. 147-153

Hohler D, (1998). Ochratoxin A in food and feed occurence, legislation and mode of action. Ernahrungswissenchaft, 37, 2-12.

Hubert, G. Y. J., Julienne, N., Charles, D. D., Daniel, F., Sandrine, P. T., Romain, F. F., ve Henry, A. Z. P. (2013). Antifungal potential and phytochemical analysis of extracts from seven Cameroonian plants against late blight pathogen Phytophthora infestans. International Journal of Current Microbiology and Applied Sciences, 2(5), 140-154.

Kordali, S., Usanmaz Bozhuyuk, A., Komaki, A., Ilhan, G., and Ercisli, S. (2022). Biological control of Penicillium on lemon fruits by essential oils of Satureja species. *Erwerbs-obstbau*, *64*(4), 703-715.

Kumar, A., Singh, P. P., Gupta, V., and Prakash, B. (2020). Assessing the antifungal and aflatoxin B1 inhibitory efficacy of nanoencapsulated antifungal formulation based on combination of Ocimum spp. essential oils. Int. J. Food Microbiol. 330:108766. doi: 10.1016/j.ijfoodmicro.2020.108766

Li, X., Tang, H., Yang, C., Meng, X., and Liu, B. (2019). Detoxification of mycotoxin patulin by the yeast Rhodotorula mucilaginosa. Food Control, 96, 47-52.

Lopez-Reyes, J. G., Spadaro, D., Gullino, M. L., and Garibaldi, A. (2010). Efficacy of plant essential oils on postharvest control of rot caused by fungi on four cultivars of apples in vivo. Flavour and Fragrance Journal, 25(3), 171-177.

Moake, M. M., Padilla-Zakour, O. I., and Worobo, R. W. (2005). Comprehensive review of patulin control methods in foods. Comprehensive Reviews in Food Science and Food Safety, 4(1), 8-21.M. Sajid, S. Mehmood, C. Niu, Y. Yuan, T. Yue Effective adsorption of patulin from apple juice by using non-cytotoxic heat-inactivated cells and spores of Alicyclobacillus strains Toxins, 10 (2018), p. 344.

Nguefack, J.; Tamgue, O.; Dongmo, J.; Dakole, C.; Leth, V.; Vismer, H.; Amvam Zollo, P.; Nkengfack, A. Synergistic action between fractions of essential oils from Cymbopogon citratus, Ocimum gratissimum and Thymus vulgaris against Penicillium expansum. Food Control 2012, 23, 377–383.

Pérez-Alfonso, C.O., Martínez-Romero, D., Zapata, P.J., Serrano, M., Valero, D., Castillo, S., (2012). The effects of essential oils carvacrol and thymol on growth of Penicillium digitatum and P. italicum involved in lemon decay. International Journal of Food Microbiology 158: 101-106.

Plaza, P., Torres, R., Usall, J., Lamarca, N., and Vinas, I. (2004). Evaluation of the potential of commercial post-harvest application of essential oils to control citrus decay. *The Journal of Horticultural Science and Biotechnology*, *79*(6), 935-940.

Sajid, M., Mehmood, S., Niu, C., Yuan, Y., and Yue, T. (2018). Effective Adsorption of Patulin from Apple Juice by Using Non-Cytotoxic Heat-Inactivated Cells and Spores of Alicyclobacillus Strains. Toxins10(9): 344.

Saleh, I. and Goktepe, I. (2019a) Health risk assessment of Patulin intake through apples and apple-based foods sold in Qatar. Helyon, 5(11)02754.

Saleh, I., and Goktepe, I. (2019b). The characteristics, occurrence, and toxicological effects of patulin. Food Chemical Toxicology, 129, 301-311.

Salhi, N., Mohammed Saghir, S. A., Terzi, V., Brahmi, I., Ghedairi, N., and Bissati, S. (2017). Antifungal activity of aqueous extracts of some dominant Algerian medicinal plants. BioMed research international, 2017.

Satish, S., Raveesha, K. A. ve Janardhana, G. R. (1999). Antibacterial activity of plant extracts on phytopathogenic Xanthomonas campestris pathovars. Letters in Applied Microbiology, 28, 145-147.

Sivakumar, D., & Bautista-Baños, S. (2014). A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage. Crop protection, 64, 27-37.

Soković, M., Tzakou, O., Pitarokili, D., and Couladis, M. (2002). Antifungal activities of selected aromatic plants growing wild in Greece. Food/Nahrung, 46(5), 317-320.

Steyn, P. S., and Stander, M. A. (1999). Mycotoxins with special reference to the carcinogenic mycotoxins: aflatoxins, ochratoxins and fumonisins. General and Applied Toxicology. 2nd Edition. United Kingdom: Macmillan Reference Ltd, 2145-76.

Taş, L., (2015). Elmada mavi küf etmeni Penicillium expansum'a karşı bazı bitki uçucu yağlarının etkileri / Effects of some plant essential oils against Penicillium expansum, blue mold agent of apple. Süleyman Demirel Üniversitesi / Fen Bilimleri Enstitüsü / Bitki Koruma Ana Bilim Dalı Yüksek Lisans Tezi

Tunail, N. (2000). Funguslar ve mikotoksinler. Gıda Mikrobiyolojisi ve Uygulamaları, Genişletilmiş, 2, 1-50.

Yanar, Y., Kadıoğlu, Ġ., Gökçe, A., DemirtaĢ, Ġ., Gören, N., Çam, H. ve Whalon, M. 2011. In vitro antifungal activities of 26 plant extracts on mycelial growth of Phytophthora infestans (Mont.) de Bary. African Journal of Biotechnology, 10(14), 2625-2629.

Yılmaz, A. (2012). Bazı bitki uçucu yağlarının hasat sonrası fungal meyve çürüklüğü etmenlerine karşı In vitro ve In vivo etkilerinin araştırılması. Selçuk Üniversitesi, Fen Bilimleri Ensitüsü, Doktora Tezi, Konya, 1-170.

Zabka, M., Pavela, R., and Slezakova, L. (2009). Antifungal effect of Pimenta dioica essential oil against dangerous pathogenic and toxinogenic fungi. Industrial Crops and Products, 30(2), 250-253.

Zhao et al., 2018 G. Zhao, X. Yang, T. Nisar, Y. Tian, L. Sun, X. Zhang, Y. Guo Patulin biodegradation and quality improvement of apple puree fermented with Byssochlamys nivea FF1-2 Food Bioscience, 21 (2018), pp. 45-52.

USE OF *ORIGANUM* SPECIES ESSENTIAL OILS IN THE CONTROL OF PLANT DISEASES

Filiz ÜNAL[1], Aysun CAVUSOGLU[2], Gülsüm Ebru ÖZER UYAR[2]

[1]Department of Plant Protection, Faculty of Agriculture, Osmangazi University, Eskişehir, Türkiye

[2]Department of Plant Protection, Faculty of Agriculture, Kocaeli University, Kocaeli, Türkiye

Corresponding author e-mail: filiz.unal@ogu.edu.tr

ABSTRACT

Origanum is a significant medicinal and aromatic plant belonging to the Lamiaceae family. In parallel with the developments in studies on the isolation and determination of various bioactive substances contained in *Origanum* species, it has been determined that they contain many different compounds. The main groups of these compounds are terpenoids, triterpene acids, hydroquinones, flavonoids, phenolic acids, hydrocarbons containing carvacrol, thymol, and various extracts as the main bioactive components. Studies on the effects of these different components against plant diseases have been the focus of attention of researchers in recent years. In studies on the impact of essential oils obtained from *Origanium* species on plant diseases, the effects of essential oils obtained from *Origanum vulgare, O. compactum, O. heracleoticum, O. onites, O. syriacum, O. dictamnus, O. acutidens, O. rotundifolium,* and *O. majorana* species were mostly in investigated. Essential oils obtained from *Origanum* species are used as antifungal agents against *Alternaria* spp, *Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Fusarium* spp., *Phytophthora* spp., *Pythium* spp., *Colletotrichum* spp., *Monilinia* spp., *Geotrichum citri-aurantii, Verticillium dahliae, Botrytis cinerea, Plasmopara viticola, Sclerotium rolfsii, Rhizoctonia solani, Macrophomina phaseolina, Sclerotinia sclerotiorum, Phoma tracheiphila, Stemphylium beticola* and *Aphanomyces euteiches.* They also have been observed to be effective as antibacterials in research on *Clavibacter* spp., *Xanthomonas* spp., *Pseudomonas spp., Erwinia spp., Ralstonia solanacearum and Pectobacterium carotovorum. The* essential oils from the *O. vulgare* plant were effective against *Zucchini yellow mosaic virus* coat protein (ZYMV-CP) and tomato leaf curl New Delhi virus (ToLCNDV) infections. Further studies are needed to evaluate the efficacy of the model products of these essential oils.

Key words: *Origanum* spp., antifungal, antibacterial, antiviral, plant diseases

INTRODUCTION

Origanum is an important medicinal and aromatic plant belonging to the Lamiaceae family. In parallel with the developments in studies on the isolation and determination of various bioactive substances contained in Origanum species, it has been determined that they contain many different compounds. The main groups of these compounds are terpenoids, triterpene acids, hydroquinones, flavonoids, phenolic acids, hydrocarbons containing carvacrol, thymol and various extracts as the main bioactive components. Studies on the effects of these different components against plant diseases have been the focus of attention of researchers in recent years.

Essential oils (EO) are aromatic oily liquids obtained from various plants, generally localized in temperate and hot countries. Essential oils, as secondary metabolites in nature, play an important role in the protection of plants as antibacterial, antiviral, antifungal and insecticide (Bakkali et al., 2008).

As observed from antifungal trials in different literature, essential oils have a great antifungal effect on many plant pathogens and have been determined to inhibit most of the plant pathogens tested in the laboratory (Omar and Kordali, 2019).

Origanum is an important medicinal and aromatic plant belonging to the Lamiaceae family. The fact that the plants of the Lamiaceae family are the source of many essential oils used in medicine and perfumery and are used both in the treatment of diseases and as spices shows the importance of this family. The effective results obtained in studies on the effects of these essential oils on plant diseases have also become the focus of agricultural research in recent years.

Thymus, Origanum, Satureja, Tymbra and Coridothymus are among the most important plants of the Lamiaceae family. The main components of the essential oils of these genera (with some exceptions) are usually carvacrol or thymol or both. One of the most important of these genera is Origanum, and 23 species of this genus grow naturally in our country. There are 41 species of this genus in the world.

EFFECT of *Origanum* spp. ESSENTIAL OILS AGAINST FUNGAL DISEASES

Guzmán-Guzmán et al. (2003) reported that the preventive effect of various concentrations of peppermint, eucalyptus, laurel, clove, sweet marjoram, rosemary, cinnamon, pepper, grapefruit and origanum spp. essential oils (1,250, 2,500 and 3,750 ppm) was possible to control *Fusarium oxysporum* f. sp. *phaseoli.* Eight substances, three of which are the main components of essential oils, from Mediterranean aromatic plants (*Verbena officinalis*, *Thymus vulgaris* and *Origanum vulgare*), were tested in vitro against some phytopathogenic fungi and postharvest fruit rot agents such as *Botrytis cinerea, Penicillium italicum, P. expansum, Phytophthora citrophthora* and *Rhizopus stolonifer*, which were previously found to be active against them. The tested compounds, β-phellandrene, β-pinene, camphene, carvacrol, citral, o-cymene, γ-terpinene and thymol exhibited fungicidal activity (Camele et al., 2012). The essential oil and various extracts obtained from *Origanum acutidens* and methanol extracts were evaluated for their antioxidative, antimicrobial and antiviral properties obtained from callus cultures (MeOH). In particular, the essential oil exhibited strong antimicrobial activity with a significant inhibitory effect against 27 of 35 bacteria (77%), 12 of 18 fungi (67%) and tested. GC and GC-MS analyses resulted in the identification of 38 components of the oil, the main component being carvacrol. MeOH Extracts obtained from plant parts showed better antioxidative activity than butylated extracts (Sökmen et al., 2004). *Origanum onites* and other essential oils were evaluated for their antibacterial and antifungal properties. The investigated oils showed antimicrobial activity and provided antifungal activity at a high concentration of 200 μg/mL. The fungi that *Origanum onites* is active against can be listed as follows; *Colletotrichum acutatum, C. fragariae and C. gloeosporioides* (Altintas et al., 2013). 83.37% carvacrol was observed as the main component in the essential oil obtained from *Origanum vulgare*. Germination of *Aspergillus flavus, A. parasiticus, A. terreus, A. ochraceus, A.*

fumigatus and *A. niger* agents was inhibited by the obtained oil (Carmo et al., 2008; Mitchell et al., 2010). *Origanum vulgare* essential oil was found to be effective against *Colletotrichum gloeosporoides, F. oxysporum, Rhizoctonia solani, P. ultimum, Botrytis cinera* (Sun et al., 2007) (Table 1).

Plant essential oils can potentially replace synthetic fungicides in the management of postharvest fruit and vegetable diseases. This study evaluated the in vitro and in vivo activities of thymol, carvacrol, linalool and trans-caryophyllene, the single components of *Origanum vulgare* essential oil, against *Monilinia laxa, M. fructigena* and *M. fructicola*, which are important phytopathogens and causative agents of brown rot in pome and stone fruits before and after harvest. Furthermore, the possible phytotoxic activities of these components were evaluated and their minimum inhibitory concentrations (MICs) were determined. In vitro experiments showed that thymol and carvacrol had the highest inhibitory concentrations. The results of in vivo experiments confirmed the potent activity of thymol and carvacrol against brown rot of peach fruits. The MIC value of thymol was found to be 0.16 lg/lL against *M. laxa* and *M. fructigena* and 0.12 lg/lL against *M. fructicola*, while for carvacrol it was found to be 0.02 lg/lL against the first two *Monilinia* species and 0.03 lg/lL against the third. The results of this study showed that thymol and carvacrol can be used after appropriate formulation to control postharvest fruit diseases caused by the examined *Monilinia* species (Elshafie et al., 2015). In addition, the oil obtained from the *Origanum compactum* plant, which contains carvacrol and thymol in its components, was found to be effective against *Alternaria alternata, Bipolaris oryzae, Fusarium graminearum, Fusarium equiseti, Fusarium verticillioides* agents (Santamarina et al., 2015) (Table 1).

It has been found to be quite successful against *Origanum majorana, Aspergillus niger, Monilinia fructicola, Penicillium expansum*. Its components include terpinen-4-ol, δ-2-carene, y-terpinene, carvacrol (Della Pepa et al., 2019). *Origanum acutidens* and its components are carvacrol, thymol, p-cymene; In vitro studies against *Alternaria alternata, A. solani, Botrytis* sp., *Fusarium acuminatum, F. culmorum, F. equiseti, F. nivale, F. oxysporum, F. sambucinum, F. semitectum, F. solani, Monilinia* sp., *Pythium ultimum, Phytophthora capsici, Rhizoctonia solani, Sclerotinia minor, Verticillium dahliae* have shown that thymol and carvacrol, which contain free hydroxyl groups in the p-cymene skeleton, are more fungitoxic than p-cymene (Kordali et al., 2008) (Table 1).

In a study, an alternative to the synthetic fungicides used was sought. *Botrytis cinerea*, a destructive fungal pathogen that causes gray mold disease in tomatoes. Antifungal activities of essential oils obtained from the aboveground parts of aromatic plants belonging to the Lamiacea family. Origanum (*Origanum syriacum* L. var. *bevanii*), lavender (*Lavandula stoechas* L. var. *stoechas)* and rosemary (*Rosmarinus officinalis* L.) were investigated against *B. cinerea*. It was found that the contact and volatile phase effects of different concentrations of essential oils inhibited the growth of *B. cinerea* in a dose-dependent manner. It was found that the volatile phase effects of essential oils were more effective on fungal growth than the contact phase effect. It was found that the volatile vapor of Origanum oil in air at 0.2 μg/ml completely inhibited the growth of *B. cinerea* (Soylu et al., 2010).

Plasmopara viticola is the most important pathogen in vine cultivation. Alternatives to reduce fungicides are needed to ensure a fully sustainable vineyard ecosystem, consumer health and public acceptance. Essential oils are among the most promising natural plant protection alternatives and have demonstrated antibacterial, antiviral and antifungal properties in various products. In the study, grape leaves were infected with *P. viticola* and then continuously fumigated. *Origanum vulgare* essential oil was proven to be sufficient to reduce the development of the pathogen by 95% 24 hours after infection during the steam treatment (Rienth et al., 2019) (Table 1).

Legumes products such as chickpeas, lentils and dry peas are widely grown for human and animal consumption. It is quite difficult to control pathogens that cause yield losses. Alternative methods should be resorted to due to the negative effects of fungicides used on human and environmental health. Safe and environmentally friendly plant-derived essential oils have been reported to be effective against some pathogenic fungi. Growth in essential oil supplemented growth media and inverted petri plate assay were used to determine the effects of 38 oils and their volatiles on mycelial growth and spore germination of important pathogenic fungi: *Aphanomyces euteiches, Botrytis cinerea, Colletotrichum lentis, Didymella pisi, D. rabiei, D. lentis, Fusarium avenaceum, Stemphylium beticola, Sclerotinia sclerotiorum* and *Pythium sylvaticum.* Addition of palmarosa, thyme, clove, cinnamon, lemongrass, citronella and oregano oils to the media inhibited the mycelial growth of all pathogens by 100% at 1:1,000 to 1:4,000 dilutions (Parikh et al., 2021) (Table 1).

The antioxidant and antimicrobial activities of methanol or dichloromethane extracts obtained from wild and organic culture samples of *Origanum dictamnus* were determined. Rancimate and malondialdehyde (MDA) were used to measure the antioxidant activity by HPLC methods in comparison with common commercial antioxidants butylated hydroxytoluene (BHT) and α-tocopherol. Extracts showing high antioxidant activity were encapsulated in liposomes and their antioxidant activity was estimated again by differential scanning calorimetry (DSC). Thermal-oxidative decomposition of samples (pure liposomes and encapsulated extracts) and modification of the main transition temperature for lipid mixture and splitting of the calorimetric peak in the presence of antioxidants were also investigated by DSC method. All extracts showed antioxidant and antimicrobial activities. Their effects were proved to be superior to α-tocopherol. Methanol extract (240 ppm) of organically grown *O. dictamnus* showed higher activity than butylated hydroxytoluene. After encapsulation in liposomes, antioxidant and antimicrobial activities were proven to be higher than the same extracts in pure form (Gortzi et al., 2007) (Table 1).

In the study, in vitro antifungal activities of essential oils and their main components (carvacrol, trans-etol and 1,8-cineole) obtained from taxonomically different medicinal plants such as thyme (*Origanum syriacum* L.), fennel (*Foeniculum vulgare* Mill.) and laurel (*Laurus nobilis* L.) growing naturally in different regions of Hatay province were investigated. The effectiveness of essential oils investigated against soil-borne fungal disease agents of bean plants, *Sclerotium rolfsii, Rhizoctonia solani* and *Macrophomina phaseolina,* has been determined to be quite high (Türkölmez and Soylu, 2014) (Table 1).

EFFECT of *Origanum* spp. ESSENTIAL OILS AGAINST BACTERIAL DISEASES

Origanum heracleoticum, whose main component is carvacrol, has been found to be effective against fungal agents such as *Aspergillus niger*, *Botrytis cinerea*, *Monilinia fructicola*, *Penicillium expansum* and bacterial agents such as *Clavibacter michiganensis, Xanthomonas campestris, Pseudomonas fluorescens, Pseudomonas syringae* pv. *phaseolicola* (Della Pepa et al., 2019). *Clavibacter michiganensis* subsp. *michiganensis* (Smith) Davis et al. (Cmm) and *Ralstonia solanacearum* Yabuuchi et al. (Smith) (Rs) are important seed-borne bacterial pathogens of tomato (Solanum lycopersicum) listed as A2 pests in the EPPO (European and Mediterranean Plant Protection Organization) region. Currently, there are few strategies to control these pathogens and seed control with eco-compatible approaches is widely promoted. In this study, essential oils (EOs) of oregano (*Origanum vulgare*), garlic (*Allium sativum*), basil

(*Ocimum basilicum*), cinnamon (*Cinnamomum zeylanicum*), clove buds (*Syzygium aromaticum*), thyme (*Thymus vulgaris)* were tested in vitro for their antimicrobial activities against Cmm and Rs. The tested essential oils caused significant inhibition of bacterial growth with very promising MBC (minimum bactericidal concentration) and MIC90 (minimum inhibitory concentration that causes 90% growth inhibition) values. Furthermore, in vivo germination test showed that there was no significant reduction in seed germination when the substances were applied as seed treatments. To test the antimicrobial activity of thyme and cinnamon oil in seed treatment in vivo, a rapid molecular screening method was developed for the specific quantification of Cmm in the presence of a plant matrix by real-time PCR without resorting to time- and space-consuming whole plant experiments (Orzali et al., 2020) (Table 1).

Hydrodistilled essential oils from *Origanum acutidens, O. rotundifolium* and *O. vulgare*. showed a broad spectrum of antibacterial activity on *Xanthomonas axonopodis*. At the same time, other main components such as carvacrol, thymol and terpinen-4-ol and linalool were found to have antimicrobial activity (Dadaşoğlu et al. 2011) (Table 1).

In vitro activity of *Origanum heracleoticum* L. essential oil compounds was tested against *Verticillium dahliae, Fusarium oxysporum, Phoma tracheiphila, Phytophthora cactorum* and *Botrytis cinerea* using four different concentrations (1×104 to 1×10-2 µg.ml-1) by well diffusion method. Carvacrol and plain essential oil showed inhibitory effect against all tested pathogens at higher concentrations (Salomone et al., 2008). In this study, essential oils of four aromatic plants were tested. The compositions and antimicrobial effects of the essential oils of cinnamon (*Cinnamomum zeylanicum*), thyme (*Thymus vulgaris*), oregano (*Origanum vulgare*) and clove (*Syzygium aromaticum*) were investigated against plant pathogenic bacteria (*Pectobacterium* spp. and *Pseudomonas* spp.). Both are commonly associated with diseased fruit trees. As a result, it was concluded that some essential oils can be used for the control of postharvest bacterial pathogens. The findings of the present study showed that essential oils have the potential to be used as antibacteial agents (Božik et al., 2017) (Table 1).

Erwinia amylovora was found to be susceptible to essential oils obtained from *Melissa officinalis, Mentha arvensis, Origanum compactum, O. vulgare, Thymus vulgaris, Eugenia caryophyllata, Mentha pulegium* plants (Kokoškova and Pavela, 2007) (Table 1).

EFFECT OF *Origanum* spp. ESSENTIAL OILS AGAINST VIRAL DISEASES

A study aimed to evaluate the antiphytoviral activity of applications with three essential oils obtained from *Origanum vulgare, Thymus vulgaris* and *Rosmarinus officinalis* and their corresponding hydrosols on *Zucchini Yellow Mosaic Virus* and *Tomato Leaf Curl New Delhi Virus* infected squash plants. Applications were applied after or simultaneously with virus inoculation to detect an inhibition or curative activity, respectively. Symptoms were observed and weekly samplings were done. Virus titer and expression levels of phenylalanine ammonia lyase gene (PAL) were measured in treated and untreated infected plants by real-time PCR. PAL gene plays an important role in plant defense response as it is involved in tolerance/resistance to phytopathogens. A significant inhibition was observed with *O. vulgare* essential oil and hydrosol, resulting in a 10-4 fold reduction in virus titer after 3 weeks of treatment (Taglienti et al., 2022) (Table 1).

Table 1. *Origanum* species found effective in controling plant diseases and the pathogens they affect

Origanum sp. from which essential oil is obtained	**Fungal Agent**	**Bacterial Agent**	**Viral Agent**	**References**
Origanum spp.	*Fusarium oxysporum f. sp. phaseoli*			Guzmán-Guzmán et al., 2003
Origanum vulgare	*Botrytis cinerea* *Penicillium italicum* *Penicillium expansum* *Phytophthora citrophthora* *Rhizopus stolonifer*			Camele et al., 2012
Origanum acutidens	*Alternaria solani* *Aspergillus flavus* *Aspergillus niger* *Fusarium oxysporum* *Fusarium solani* *Moniliania fructicola* *Penicillum spp.* *Rhizopus spp.* *Rhizoctonia solani* *Sclorotinia sclerotiorum*	*Clavibacter michiganense* *Pseudomonas syringae* pv.tomato *Xanthomonas campestris*		Sökmen et al.,2004
Origanum onites	*Colletotrichum acutatum* *Colletotrichum fragariae* *Colletotrichum gloeosporioides*			Altintas et al.,2013
Origanum vulgare	*Aspergillus flavus* *Aspergillus parasiticus* *Aspergillus terreus* *Aspergillus ochraceus* *Aspergillus fumigatus* *Aspergillus niger*			Carmo et al., 2008 Mitchell et al., 2010
Origanum vulgare	*Colletotrichum gloeosporoides* *Fusarium oxysporum,* *Rhizoctonia solani* *P.ultimum* *Botrytis cinera* *Monilinia laxa* *M..fructigena* *M. fructicola*			Sun et al., 2007 Elshafie et al., 2015
Origanum compactum	*Alternaria alternata* *Bipolaris oryzae* *Fusarium graminearum* *Fusarium equiseti* *Fusarium verticillioides*			Santamarina et al., 2015

Origanum majorana,	*Aspergillus niger* *Monilinia fructicola* *Penicillium expansum*			Della Pepa et al., 2019
Origanum acutidens	*Alternaria alternata* *Alternaria solani* *Botrytis* sp. *Fusarium acuminatum* *Fusarium culmorum* *Fusarium equiseti* *Fusarium nivale* *Fusarium oxysporum* *Fusarium sambucinum* *Fusarium semitectum* *Fusarium solani* *Monilinia sp.* *Pythium ultimum* *Phytophthora capsici* *Rhizoctonia solani* *Sclerotinia minör* *Verticillium dahliae*			Kordali *et al*.,2008
Origanum syriacum	*Botrytis cinerea*			Soylu et al., 2010
Origanum vulgare	*Plasmopara viticola*			Rienth et al., 2019
Origanum vulgare	*Aphanomyces euteiches* *Botrytis cinerea* *Colletotrichum lentis* *Didymella pisi,* *Didymella rabiei* *Didymella lentis* *Fusarium avenaceum* *Stemphylium beticola* *Sclerotinia sclerotiorum* *Pythium sylvaticum*			Parikh et al., 2021
Origanum syriacum	*Sclerotium rolfsii* *Rhizoctonia solani* *Macrophomina phaseolina*			Türkölmez ve Soylu, 2014).
Origanum heracleoticum	*Aspergillus niger* *Botrytis cinerea* *Monilinia fructicola* *Penicillium expansum*	*Clavibacter michiganensis* *Xanthomonas campestris* *Pseudomonas fluorescens* *Pseudomonas syringae pv. phaseolicola*		Della Pepa et al., 2019
Origanum vulgare		*Clavibacter michiganensis subsp. Michiganensis* *Ralstonia solanacearum*		Orzali et al., 2020

Origanum acutidens *O. rotundifolium* *O. vulgare*		*Xanthomonas axonopodis*		Dadaşoğlu ve ark. 2011
Origanum heracleoticum	*Verticillium dahliae, Fusarium oxysporum, Phoma tracheiphila, Phytophthora cactorum Botrytis cinerea*			Salomone et al., 2008
Origanum vulgare		*Pectobacterium spp*. *Pseudomonas spp*.		Božik et al., 2017
Origanum compactum *O. vulgare,*		*Erwinia amylovora*		Kokoškova ve Pavela, 2007
Origanum vulgare			*Zucchini Yellow Mosaic Virus, Tomato Leaf Curl New Delhi Virus*	Taglienti et al., 2022

CONCLUSIONS

In recent years, interest in the biological activities of natural compounds has been increasing due to the increasing use of fungicides that pose a risk to human and environmental health. In plant protection, essential oils are reported to exhibit antiviral, antifungal and antibacterial activities and are considered promising for the formulation of safe antimicrobial agents. Essential oils are considered as an alternative method due to their low cost and availability, and further research is required to make their use widespread.

REFERENCES

Altintas, A., Tabanca, N., Tyihák, E., Ott, P. G., Móricz, Á. M., Mincsovics, E., ve Wedge, D. E. (2013). Characterization of volatile constituents from *Origanum* onites and their antifungal and antibacterial activity. *Journal of AOAC International*, *96*(6), 1200 1208.

Bakkali, F., Averbeck, S., Averbeck, D., ve Idaomar, M. (2008). Biological effects of essential oils–a review. *Food and chemical toxicology*, *46*(2), 446-475.

Božik, M., Nový, P., ve Klouček, P. (2017). Chemical composition and antimicrobial activity of cinnamon, thyme, oregano and clove essential oils against plant pathogenic bacteria. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, *65*(4).

Camele, I., Altieri, L., De Martino, L., De Feo, V., Mancini, E., ve Rana, G. L. (2012). In vitro control of post-harvest fruit rot fungi by some plant essential oil components. *International journal of molecular sciences*, *13*(2), 2290-2300.

Carmo, E.S.,Lima, E.,Souza, E.L., 2008. The potential of *Origanum vul- gare* L. (Lamiaceae) essential oil in inhibiting the growth of some food-related *Aspergillus* species. Braz J Microbiol, 39: 362–367.

Dadasoglu F, Aydin T, Kotan R et al (2011) Antibacterial activities of extracts and essential oils of three *Origanum* species against plant pathogenic bacteria and their potential use as seed disinfectants. J Plant Pathol 93(2):271–282.

Della Pepa T, Elshafie HS, Capasso R, De Feo V, Camele I, Nazzaro F, Scognamiglio MR, Caputo L. 2019. Antimicrobial and phytotoxic activity of *Origanum* heracleoticum and O. majorana essential oils growing in Cilento (Southern Italy). Molecules 24(14):2576

Elshafie, H. S., Mancini, E., Sakr, S., De Martino, L., Mattia, C. A., De Feo, V., ve Camele, I. (2015). Antifungal activity of some constituents of *Origanum* vulgare L. essential oil against postharvest disease of peach fruit. *Journal of Medicinal Food*, *18*(8), 929-934.

Ghalem, B. R. (2016). Essential oils as antimicrobial agents against some important plant pathogenic bacteria and fungi. *Plant-Microbe Interaction: An Approach to Sustainable Agriculture*, 271-296.

Gortzi, O., Lalas, S., Chinou, I., ve Tsaknis, J. (2007). Evaluation of the antimicrobial and antioxidant activities of *Origanum* dictamnus extracts before and after encapsulation in liposomes. *Molecules*, *12*(5), 932-945.

Guzmán-Guzmán A, Navarrete-Maya R, Navarrete-Maya J (2003) In vitro control of *Fusarium oxysporum* f sp. *phaseoli* with vegetal essential oils. XLVI Report Bean Improv Cooper 46:221–222.

Kokoškova B, Pavela R (2007) Effectiveness of plant essential oils on the growth of *Erwinia amylovora*, the causal agent of Fire Blight Disease. Pest Technol 1(1):76–80.

Kordali, S., Cakir, A., Ozer, H., Cakmakci, R., Kesdek, M., ve Mete, E. (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum* acutidens and its three components, carvacrol, thymol and p-cymene. *Bioresource technology*, *99*(18), 8788-8795.

Mitchell, T.C., Stamford, T.L.M.,Souza, E.L.D.,Lima, E.D.O.,Carmo, E.S., 2010. *Origanum vulgare* L. essential oil as inhibitor of potentially toxigenic *Aspergilli* . Cienc Tecnol Aliment, 30: 755–760.

Omar, M. S., ve Kordali, Ş. (2019). Review of essential oils as anti-fungal agents for plant fungal diseases. *Ziraat Fakültesi Dergisi*, *14*(2), 294-301.

Orzali, L., Valente, M. T., Scala, V., Loreti, S., ve Pucci, N. (2020). Antibacterial activity of essential oils and Trametes versicolor extract against Clavibacter michiganensis subsp. michiganensis and Ralstonia solanacearum for seed treatment and development of a rapid in vivo assay. *Antibiotics*, *9*(9), 628.

Parikh, L., Agindotan, B. O., ve Burrows, M. E. (2021). Antifungal activity of plant-derived essential oils on pathogens of pulse crops. *Plant Disease*, *105*(6), 1692-1701.

Rienth, M., Crovadore, J., Ghaffari, S., ve Lefort, F. (2019). Oregano essential oil vapour prevents Plasmopara viticola infection in grapevine (Vitis Vinifera) and primes plant immunity mechanisms. *PLoS One*, *14*(9), e0222854.

Salomone, A., Scaritto, G., Sacco, I., Cabras, P., ve Angioni, A. (2008). Inhibitory effects of the main compounds of oregano essential oil against some pathogenic fungi.

Santamarina MP, Rosello J, Sempere F, Gimenez S, Blazquez MA. 2015. Commercial *Origanum* compactum Benth. and Cinnamomum zeylanicum Blume essential oils against natural mycoflora in Valencia rice. Natural Product Research 29(23):2215–2218

Sökmen, M., Serkedjieva, J., Daferera, D., Gulluce, M., Polissiou, M., Tepe, B., ... ve Sokmen, A. (2004). In vitro antioxidant, antimicrobial, and antiviral activities of the essential oil and various extracts from herbal parts and callus cultures of *Origanum* acutidens. *Journal of agricultural and food chemistry*, *52*(11), 3309-3312.

Soylu, E. M., Kurt, Ş., ve Soylu, S. (2010). In vitro and in vivo antifungal activities of the essential oils of various plants against tomato grey mould disease agent Botrytis cinerea. *International journal of food microbiology*, *143*(3), 183-189.

Sun, O.L.,Gyung, J.C.,Kyoung, S.J.,He, K.L.,Kwang, Y.C.,Jin-Cheol, K., 2007. Antifungal activity of five plant essential oils as fumigant against postharvest and soil borne plant pathogenic fungi. Plant Path J, 23: 97–102 .

Taglienti, A., Donati, L., Ferretti, L., Tomassoli, L., Sapienza, F., Sabatino, M., ... ve Ragno, R. (2022). In vivo antiphytoviral activity of essential oils and hydrosols from *Origanum* vulgare, Thymus vulgaris, and Rosmarinus officinalis to control zucchini yellow mosaic virus and tomato leaf curl New Delhi virus in Cucurbita pepo L. *Frontiers in Microbiology*, *13*, 840893.

Türkölmez, S., ve Soylu, E. M. (2014). Antifungal efficacies of plant essential oils and main constituents against soil-borne fungal disease agents of bean. *Journal of Essential Oil Bearing Plants*, *17*(2), 203-211.

PHENOLOGICAL AND MORPHOLOGICAL DEVELOPMENT OF DIFFERENT CULTIVARS OF GREEN BEANS

Dimka Haytova[1]

[1]*Agricultural University, Faculty of viticulture and horticulture Department of Horticulture, Plovdiv, Bulgaria*

Corresponding author e-mail: haitova@abv.bg

ABSTRACT

It is necessary to extend the period of supply of fresh products and raw materials for processing, which is related to the adaptation of sowing and planting periods of vegetable crops to the changed environmental situation.
Green beans are an important vegetable crop essential for nutritional balance and solving the protein problem for many peoples of the world.
The main reason for the development of this paper is the establishment and popularization of up-to-date scientific information on the phenological and morphological development of garden beans, with a view to expanding the range of varieties in this vegetable crop.
The experiments were carried out in the period 2021-2022, in the area of the village of Ezero, Nova Zagora municipality, with an altitude of 131 m, located in the Thracian valley. Four varieties of green beans were used. The experimental design was based on the block method. A total of 15 variants were tested, which were a combination of each variety and the three sowing dates.
The results show that late sowing (May) contributes to the formation of better biometric indicators, supporting better vegetative development of plants and the establishment of an actively photosynthesizing leaf apparatus. By sowing at the beginning of May, the plants develop under conditions closer to their biological requirements and a better synchronization between vegetative development, flowering and fruiting is achieved.

Keywords: garden beans, *Phaseolus vulgaris*, development, growth

INTRODUCTION

Green beans are an important vegetable crop, essential for nutritional balance and solving the protein problem for many peoples of the world. In the context of a healthy and rational diet for the population, there is a growing need to extend the period for the supply of fresh produce and raw material for processing, which is linked to adapting the sowing and planting times of vegetable crops to the changed environmental condition.

A detailed description of all stages of plant growth and development was made by Shaban (2014). The author defines two main phases of the bean life cycle: vegetative and reproductive. The vegetative phase lasts from seed germination until the first flower bud appears. During this phase, the stem and branches form nodes with lateral buds from which vegetative and generative organs later develop.

The reproductive phase begins with the appearance of the first flower and continues until physiological maturity. In determinate forms, growth ends with the differentiation of the apical flower bud, while in indeterminate forms it continues through the reproductive phase. The two phases are divided into 10 periods, the beginning and end of which are determined by easily identifiable physiological events that occur in all plants, regardless of their habit and the influence of external conditions. The first five periods (germination, emergence, first true leaf, first triple leaf) belong to the vegetative phase and the remaining periods (budding, flowering, bean growth, grain growth and maturity) to the reproductive phase.

The duration of periods is affected differently by genotype and environment and their interaction. Under the same conditions, the periods of germination, emergence, simple leaves hardly differ among varieties. However, they are subject to considerable variability depending on changes in environmental conditions. Recently, studies in this country have shown that the length of the collection period from germination to flowering decreases when sowing is delayed from mid-April to the end of May and increases when sowing is further delayed until mid-July. A decrease in the length of the period coincides with an increase in the mean daily temperature and a decrease in the relative humidity and, conversely, a lengthening of the growing season coincides with a decrease in temperature and an increase in the relative humidity. The total length of the flowering to maturity periods is equally influenced by environmental conditions, and with a delay in sowing from early May to mid-July, it becomes longer, coinciding with a decrease in temperature, an increase in relative humidity and a decrease in day length (Shaban, 2014).

The need to optimize sowing dates and determine their influence on the growth performance of different garden bean varieties is a subject of scientific interest by scientists worldwide. The effects of cultivar and growing season on growth development and fruit yield of garden beans were analyzed in a two-year experiment in Spain by Pérez-Barbeito et al. (2008). The effects of seed size and sowing dates on green bean growth and yield were the subject of experiments conducted by Nosser and Behnan (2011). Elhag and Hussein (2014) studied the effect of sowing date on plant development and yield. Mashiqa et al. (2019) investigated the growth performance of different genotypes of garden bean depending on sowing date.

Similar research has been conducted with other vegetable crops such as Haque et al (2013) - potato; Dhaliwal et al. (2017) - pepper, Prosanta et al. (2013) - okra, Yaser et al. (2016) - garlic.

The main aim of this publication is to comparatively evaluate the phenological development and vegetative performance of garden bean varieties at different sowing dates.

MATERIAL AND METHOD

The research was carried out in the period 2021-2022, in the land of the village of Ezero, Nova Zagora municipality, with an altitude of 131 m., located in the Thracian valley, near the Mountain Sredna Gora from the north and the Iliyskite hills in the southeast.

The prevailing topography is flat, the river Blatnitsa runs through the land. The soil is black earth, heavy, clayey, difficult to warm. Climatic conditions. Snow cover is very rare. Precipitation during the year is insufficient. Summers are relatively hot and droughts are frequent. In recent years there has been an almost constant summer dry spell.

The experiment was designed using the block method in four replications with an experimental plot size of 10 m^2 and a treatment plot size of 8 m^2.

For the purpose of the experiment the varieties 'Capitano', 'Gina', 'Ebro', 'Playa' 'Bergold' were used. All varieties are determinate, with a bush habitus, suitable for fresh consumption and for processing.

A total of 15 variants were tested, which were a combination of each variety and the three sowing dates. Seeds of the five varieties were sown on three different sowing dates, 10 days apart, during the period from the middle of April to the first ten days of May (13.04; 24.04; 03.05, respectively).

The plants were grown according to the technology adopted for southern Bulgaria for growing garden beans (Cholakov, 2009). The cultivation scheme was 60 x 20 cm. Irrigation was done by drip irrigation system. For the optimal development of the plants, the necessary agronomic practices, based on the applied technology, were carried out during the growing season. Harvesting was done manually when the fruits reached consumption maturity (Mihov et al., 2014).

Phenological observations were made to determine the phases mass: germination (in days after sowing), appearance of: first true leaf, first triple leaf, budding, flowering, fruit formation, fruiting. The mass occurrence of the respective phase was recorded in 70% of the plants.

At mass flowering and mass fruiting stage, morphological characterization of plants including: stem height (cm); stem weight (g); number of leaves; leaf weight (g); number of blossoms, number of fruits, weight of fruits was determinate on 5 plants of each replicate;

RESULTS AND DISCUSSION

Different sowing dates of garden beans (Table 1) affect seed germination. Seeds sown at the earliest sowing date take the longest time to germinate, ranging from 16 to 21 days, with little variation between varieties. When sown in the middle of April, the vegetative development of the plants is slow, as illustrated by the time taken for the first leaves to form on the first true leaf. This varies from 31 to 39 days for different varieties. The generative organs are laid down approximately one and a half months after the plants emerge. The earliest buds appear in the Capitano variety and the latest in the Playa variety. Mass flowering in the varieties tested occurs between the 51st and 70th day after germination, following the trend of the previous phase. Mass fruiting occurs from day 69 for the variety Gina to day 91 for the variety Playa. There is little difference in the onset of fruiting in the varieties Gina, Capitano and Ebro. The difference with the other two varieties is 12 and 22 days respectively. The discrepancy in the onset of mass fruiting creates favorable conditions for regular harvesting (Shaban, 2014).

Table 1. Length of interphase periods at first sowing date (13.04) /days after sowing/

variety	germination	first true leaf	first triple leaf	budding	flowering	Fruit formation	fruiting
Capitano	16	22	31	38	51	61	70
Gina	17	24	32	42	53	62	69
Ebro	20	26	33	43	54	63	74
Bergold	18	25	36	46	65	74	81
Playa	21	27	39	49	70	81	91

As temperature conditions improved under field conditions, seeds from the second date germinated, between days 11 and 15, respectively (Table 2). The first generative organs set between 45-59 days after germination. Mass fruiting also occurs without significant differences in the Capitano, Gina and Ebro varieties. The Bergold and Playa varieties come into fruit at 70 and 80 days after germination respectively.

Table 2. Length of interphase periods at second sowing date (24.04) /days after sowing/

variety	germination	first true leaf	first triple leaf	budding	flowering	Fruit formation	fruiting
Capitano	11	17	26	30	45	55	64
Gina	12	19	27	37	48	59	66
Ebro	17	23	30	34	45	56	67
Bergold	13	20	29	35	54	63	70
Playa	15	23	30	38	59	70	80

A similar trend in the progression of interphase periods was observed for sowing in the beginning of May (Table 3).

Table 3. Length of interphase periods at third sowing date (03.05) /days after sowing/

variety	germination	first true leaf	first triple leaf	budding	flowering	Fruit formation	fruiting
Capitano		15	24	28	41	51	60
Gina	10	17	25	35	46	55	62
Ebro	14	20	27	31	42	51	62
Bergold	10	17	26	32	51	60	67
Playa	13	19	26	34	55	66	76

The results obtained show a harmonious progress of the interphase periods without abrupt deviations from normal plant development. They are in line with the development process of garden bean described by Shaban (2014). The length of the harvest period from emergence to flowering decreases when sowing is delayed from the middle of April to the end of May. Decrease in period length coincides with increase in mean daily temperature and decrease in relative humidity and conversely, increase in growing season length coincides with decrease in temperature and increase in relative humidity.

Biometric measurements to investigate the vegetative development of the plants were carried out twice during the vegetation in the mass flowering phase (Table 4) and in the mass fruiting phase (Table 5).

The first biometric measurement of the plants showed small differences in stem height at the three sowing dates. The same trend is also observed in the formation of flowers and setting of the first fruits.

Table 4. Biometric parameters of garden bean plants at mass flowering stage

variety	stem height (cm)	stem weight (g)	numbe r of leaves	leaf weigh t (g)	number of blossoms	number of fruits	weight of fruits (g)
first sowing date (13.04)							
Capitano	9.1	14.1	9	20.1	35	6	41.5
Gina	9.9	11.8	14	18.6	30	5	27.5
Ebro	8.8	6.0	8	16.3	12	4	23.8
Bergold	4.8	9.1	12	19.7	28	4	25.9
Playa	7.5	9.3	15	20.4	19	6	29.8
second sowing date (24.04)							
Capitano	8.9	13.8	10	19.8	34	4	35
Gina	9.7	12.6	13	17.6	33	3	26
Ebro	8.5	6.4	8	15.4	10	3	25
Bergold	5.0	8.9	12	18.3	27	3	23
Playa	7.9	9.2	15	21.2	20	4	26
third sowing date (03.05)							
Capitano	9,4	14,3	11,2	21,8	36	6	48,1
Gina	10,1	12,8	15,1	19,7	35	5	39,4
Ebro	9,3	6,4	9,7	17,9	13	3	21,3
Bergold	4,5	9,4	13,8	21,1	29	4	27,6
Playa	8	9,4	14,4	22,3	21	5	17,4

Within 30 days, until the next biometric measurements, there was a slight increase in stem growth, an increase in the number of leaves and in the fresh mass of the plants. A steady flower set and fruit formation was observed (Table 5).

Table 5: Biometric parameters of garden bean plants in mass fruiting phase

variety	stem height (cm)	stem weight (g)		number of leaves	leaf weight (g)	number of blossoms	number of fruits	weight of fruits (g)
	first sowing date (13.04)							
Capitano	41.3	16.5		28	104.8	68	58	350.2
Gina	36.5	15.4		24	94.3	39	51	264.4
Ebro	32.8	9.3		19	68.9	31	20	240.0
Bergold	28.0	13.8		25	90.2	37	36	190.4
Playa	32.6	12.6		21	57.2	25	23	161.0
	second sowing date (24.04)							
Capitano	42.6	18.6		28	103.3	68	54	348.7
Gina	37.7	16.4		24	92.5	40	52	355.7
Ebro	34.3	10.9		17	64.6	30	20	140.4
Bergold	28.0	14.2		25	86.1	36	38	202.54
Playa	33.2	14.8		20	52.4	21	23	162.38
	third sowing date (03.05)							
Capitano	43.7	19.9		27	101.1	64	53	320.4
Gina	38.4	17.9		23	90.4	36	52	290.3
Ebro	35.3	11.8		16	58.6	29	15	180.4
Bergold	28.6	14.5		23	83.1	35	38	201.7
Playa	34.9	15.3		18	49.6	19	21	147.5

Good plant foliation is a precondition for the formation of a developed and active assimilation apparatus, providing an active photosynthetic surface for the normal course of physiological processes in the plant organism. It is necessary to note that in this period there are formed mass formation of fruits, which is the visible expression of the vegetative growth deceleration and the gradual transition of plants into the reproductive phase of their development (Alemu et al., 2017).

CONCLUSIONS

Late sowing (in May) contributes to the formation of better biometric indicators, justifying better vegetative development of plants and the establishment of an actively photosynthesizing leaf apparatus.
Sowing at the beginning of May results in the plants developing under conditions closer to their biological requirements and in better synchronisation between vegetative development, flowering and fruiting.

REFERENCES

Alemu, Y., Alamirew, S., & Dessalegn, L. (2017). Correlation and path analysis of green pod yield and its components in snap bean (Phaseolus Vulgaris L.) genotypes. *International Journal of Research*, *30*.

Cholakov, D., 2009. Vegetable Production, Academic Publishing House of Agricultural University – Plovdiv, pp. 150-158. (in Bulgarien)

Dhaliwal M. S., S. P. Sharma, S. K. Jindal, L. K. Dhaliwal & A. K. Gaikwad (2017) Growth and yield of bell pepper as influenced by growing environment, mulch, and planting date, Journal of Crop Improvement, 31:6, 830-846, DOI: 10.1080/15427528.2017.1391146

Elhag, A. Z., & Hussein, A. M. (2014). Effects of sowing date and plant population on snap bean (Phaseolus vulgaris L.) growth and pod yield in Khartoum State. Universal Journal of Agricultural Research, 2(3), 115-118.

Haque M.E., Hossain1 M.S, Islam M.K., M.H. Waliullah and T.K.Dey (2013) Effect of planting time and fungicidal spray in controlling late blight of potato variety Raja J. Agrofor. Environ. 7 (1): 123-126, 2013 ISSN 1995-6983

Mashiqa, P., Moatshe, O., Tiroesele, B., Lekgari, L., & Molosiwa, O. (2019). Response of common bean (Phaseolus vulgaris L.) genotypes to varying planting dates in Botswana. Journal of Agricultural and Crop Research, 7(2), 26-30.

Mihov, Kr., Panayotov, N., Filippov, St., Babrikov, T., Kostadinov, K., Haytova, D. 2014. Manual of Vegetable Production with Seed Production, Academic Publishing House of Agricultural University – Plovdiv (in Bulgarien)

NOSSER, M. A., & BEHNAN, E. Y. (2011). Effect of seed size and sowing dates on growth and yield of green and dry bean (Phaseolus vulgaris L). Egyptian Journal of Agricultural Research, 89(3), 1053-1070.

Pérez-Barbeito, M., González, A. M., Rodiño, A. P., De Ron, A. M., & Santalla, M. (2008). Effects of planting season and plant cultivar on growth, development, and pod production in snap bean (Phaseolus vulgaris L.). Australian journal of agricultural research, 59(12), 1121-1129.

Prosanta Kumar Dash, Md. Golam Rabbani, Md. Ferdous Mondal (2013) Effect of variety and planting date on the growth and yield of okra International Journal of Biosciences | IJB |, ISSN: 2220-6655 (Print) 2222-5234 (Online) ,Vol. 3, No. 9, p. 123-131, 2013 http://www.innspub.net,

Shaban N.2014. Vegetable production, Publishing house at LTU, Sofia (in Bulgarien)

Yaser A.E. Mohamed, E.A. El-Ghamriny, A. Bardisi* and Dalia A.S. Nawar EFFECT OF PLANTING DATE AND FOLIAR SPRAY WITH POTASSIUM SILICATE ON GROWTH AND YIELD OF SOME GARLIC CULTIVARS Zagazig J. Agric. Res., Vol. 46 No. (2) 2019

STUDY OF DIFFERENT SOWING DATES ON YIELD AND FRUIT QUALITY OF GREEN BEANS

Dimka Haytova[1]

[1]*Agricultural University, Faculty of viticulture and horticulture Department of Horticulture, Plovdiv, Bulgaria*

Corresponding author e-mail: haitova@abv.bg

ABSTRACT

Green beans are a vegetable crop with a very high nutritional value. Research on appropriate sowing dates and varietal structure of garden beans is topical and has been investigated by various scientists. It is topical to renew the cultivar structure of garden beans in order to expand the variety of this vegetable crop and to extend the period of fresh production on the Bulgarian market. The objective of this paper is to comparatively evaluate the cultivar characters of green bean at different sowing dates by observing the yield and fruit quality.
The experiments were carried out in the period 2021-2022, in the area of the village of Ezero, Nova Zagora municipality, with an altitude of 131 m, located in the Thracian valley. Four varieties of green beans were used. The experimental design was based on the block method. A total of 15 variants were tested, which were a combination of each variety and the three sowing dates.
The results show that higher yield was obtained from plants sown in early May. The difference with the yield from the second sowing date varies between 11 and 14%, with small differences for the different varieties. The same plants also yielded 57% to 22% higher than the first harvest. The results of the economic productivity of the plants and the quality characteristics of the fruit give us reason to conclude that the cultivars Capitano, Gina, Ebro and Playa can be recommended for cultivation with a view to enriching the cultivar range with new varieties of green beans.

Keywords: garden beans, *Phaseolus vulgaris*, cultivars, growth, yield

INTRODUCTION

Green beans are a vegetable crop with a very high nutritional and value. biological value (Cholakov, 2009; Shaban, 2014).

Asia and especially India and China are the largest producers of green beans with approximately 50% of the total area in the world, closely followed by the U.S. (FAO, 2010). In European Union countries, green bean yields are high. They are highest in Poland at 20 000 kg/ha, Spain at 14 576 kg/ha, Portugal at 13 076.9 kg/ha, Cyprus at 11 796.1 kg/ha, Austria at 11 255.7 kg/ha and Belgium at 11 494.25 kg/ha (FAO, 2010). According to the Agrostatistics Department of the Ministry of Agriculture, the area harvested in 2022 with green beans is 184 ha, average yields are 9046 kg/ha, total production amounts to 6522 tonnes (Ministry of Agriculture, 2022).

Ensuring optimal conditions for plant growth and development during the growing season are the basis for the expression of their maximum productive potential and an important prerequisite for increasing the profitability of vegetable production. In this regard, optimizing agronomic factors to manage the productivity of vegetable crops is of primary importance.

At this time, research on the appropriate sowing dates and cultivar structure of garden beans is current and is the subject of research in various countries.

Research has shown that the highest yields are obtained when sowing in late April and the lowest when sowing from 20 May to 5 June. However, over the last decade, varieties with reduced sensitivity to high temperatures have entered production, and conditions have been created for industrial crops that reduce the detrimental effects of adverse climatic factors (Kenneth, 2012).

Sowing date has significant effect on yield and yield attributes is the argument of Pandey et al. (2012). According to them, early sowing of garden bean has a positive effect on early initiation of flowering and formation of taller plants with the largest fruits The early sowing date also recorded the highest yield compared to the other two, averaging 35.35 t/ha.

Sharma et al. (2013) stated that optimum sowing time and correct cultivar selection are essential to achieve potential biological yield for garden bean.

To investigate the effect of different planting dates on yield and yield components of two garden bean varieties Mirzaienasab and Mojaddam (2014) conducted a research study over two years. They found the highest percentage of yield and yield components at the earliest sowing date and the lowest results were recorded at the latest sowing date.

The results of Rao (2022) showed that sowing dates have significant effect on total yield, fresh weight of beans fruits, length of bean fruits and total soluble solids. Higher market and non-market yields, as well as greater pod length and soluble solids, in all three varieties, were more pronounced at midle and late sowing dates.

The quality of green beans is also dependent on changes in climatic conditions, which are summarized in the different sowing dates. In all varieties, the tasting score decreases in a regular manner starting from the sowing in the middle of April until the sowing in the end of May and then starts to increase, equaling the score at the first sowing date in the middle of July (Shaban, 2014). This thesis is confirmed by Spravoli et al. (2015).

An important aspect in the light of which research on optimizing sowing times can be argued is the climate change that is occurring. Last but not least, the need to renew the cultivars composition of garden beans in order to expand the range of this vegetable crop and extend the period of fresh production on the Bulgarian market should also be noted.

The main aim of this paper is to comparatively evaluate the cultivar characters of green bean at different sowing dates by observing the yield and fruit quality.

MATERIAL AND METHOD

The research was carried out in the period 2021-2022, in the land of the village of Ezero, Nova Zagora municipality, with an altitude of 131 m., located in the Thracian valley, near the Mountain Sredna Gora from the north and the Iliyskite hills in the southeast.

The prevailing topography is flat, the river Blatnitsa runs through the land. The soil is black earth, heavy, clayey, difficult to warm. Climatic conditions. Snow cover is very rare. Precipitation during the year is insufficient. Summers are relatively hot and droughts are frequent. In recent years there has been an almost constant summer dry spell.

The experiment was designed using the block method in four replications with an experimental plot size of 10 m^2 and a treatment plot size of 8 m^2.

For the purpose of the experiment the varieties 'Capitano', 'Gina', 'Ebro', 'Playa' 'Bergold' were used. All varieties are determinate, with a bush habitus, suitable for fresh consumption and for processing.

A total of 15 variants were tested, which were a combination of each variety and the three sowing dates. Seeds of the five varieties were sown on three different sowing dates, 10 days apart, during the period from the middle of April to the first ten days of May (13.04; 24.04; 03.05, respectively).

The plants were grown according to the technology adopted for southern Bulgaria for growing garden beans (Cholakov, 2009). The cultivation scheme was 60 x 20 cm. Irrigation was done by drip irrigation system. For the optimal development of the plants, the necessary agronomic practices, based on the applied technology, were carried out during the growing season. Harvesting was done manually when the fruits reached consumption maturity (Mihov et al., 2014).

The yield was determined on the basis of three harvests carried out at the consumption stage of the fruit.

Morphological characteristics of the fruit were determined using an average sample of 20 fruit from each variant. Observations and measurements were carried out to determine: - color of the beans; - fruit length (cm); - fruit diameter (cm); - fruit weight (g);

A sensory evaluation of the fruit was carried out on average samples of each variant by interviewing 20 consumers. The evaluation was carried out on the following characteristics: size; color; texture; presence of lyco; texture; consumer preference.

RESULTS AND DISCUSSION

The results showed that the third sowing date (03.05) had the greatest positive effect on the yield of all garden bean cultivars (Table 1). For the Capitano cultivar, the increase compared to the first sowing date was 21.69% and compared to the second date - 11.25%. The yield of Gina cultivar was higher by 25.08% compared to the first date and by 14.36% compared to the second date. The increase for the Ebro cultivar was 43.31% and 14.00%, respectively, compared to the first and second sowing dates.

Table 1 Total yield and fruiting dynamics of garden bean cultivars

cultivar	First harvest kg/dka	Second harvest kg/dka	Third harvest kg/dka	Total yield kg/dka
first sowing date (13.04)				
Capitano	73.75	109.91	220.35	404.01
Gina	68.08	91.45	190.24	349.77
Ebro	42.56	37.82	120.22	200.60
Bergold	35.61	30.42	139.43	205.46
Playa	25.99	27.30	98.46	151.75
second sowing date (24.04)				
Capitano	80.60	120.79	240.34	441.73
Gina	72.14	103.49	206.90	382.53
Ebro	44.99	75.78	131.40	252.17
Bergold	39.40	97.98	151.97	289.35
Playa	30.60	73.94	109.10	213.64
third sowing date (03.05)				
Capitano	91.66	133.33	266.66	491.65
Gina	79.16	116.66	241.66	437.48
Ebro	49.99	87.50	149.99	287.48
Bergold	44.99	109.99	168.33	323.31
Playa	33.34	81.66	123.33	238.33

Sowing the Bergold cultivar in the beginning May resulted in a 57.36% higher yield compared to sowing in middle of April. For the same variety, the yield was 11.73% higher compared to that obtained from plants sown on the third tenth day of April. The differences in yields of the cultivar Playa are: an increase of 57.05% compared to the first sowing date and 11.56% compared to the yield of the second sowing date.

The increase in yield with the sowing date in the beginning of May may be due to the availability of favorable environmental conditions for appropriate timing of flowering and subsequent transformation into fruit to achieve optimum yield (Topliceanu et al., 2018).

Lower yields at early sowing dates may be associated with reduced concentrations of reducing sugars in flower buds and flowers, which may lead to pollination inefficiency and cause flower and panicle drop under sub-optimal temperature conditions for bean flowering (De Ron et al., 2016; Rao, 2022).

Alemu et al (2017) defined yield as a complex quantitative trait that is controlled by several yield-related elements, and thus information on the nature and degree of association among traits contributing to yield is useful for indirect selection to improve yield. They summarized the experiments of different researchers and reported a positive correlation between number of pods per plant and green pod yield, and reported a positive correlation between green pod length and green pod yield.

Our results are in full agreement with the conclusions of the authors, who substantiate these plant responses with more suitable environmental conditions during flowering and fruiting.

Garden bean varieties of ssp. nanos, to which those involved in the experiment belong, are characterized by amicable fruit formation resulting in three successive harvests. At each successive harvest, the quantity of fruit picked increased. The cultivars can be ranked, in descending order, as follows: Capitano, Gina, Bergold, Ebro, Playa. Capitano is the highest yielding cultivar. Playa is the lowest yielding cultivar (Table 1).

The marketability of garden beans for fresh consumption depends on the appearance of the beans and their uniformity. In this regard, measurements were carried out to determine the morphological characteristics of the fruits of the cultivars studied (Table 2).

Table 2. Morphological characteristics of beans fruits

Cultivar	Color	fruit length (cm)	fruit diameter (cm)	fruit weight (g)
Capitano	Yellow	14	1.9	7.03
Gina	Green	16	1.8	6.84
Ebro	Green	15	1.6	7.02
Bergold	Yellow	13	0.8	5.33
Playa	Green	15	1.4	7.06

Two of the cultivars have yellow colour of bean fruits and the other three have green color of beans fruits. Of all the cultivars, only the Bergold variety has cylindrical shape of fruits. The length of the fruit varies from 13 to 16 cm for different cultivars. Fruit diameter varies from 0.8 to 1.9 cm. Bergold is relatively smaller. For the other cultivars, there is little variation in weight. When the general appearance of the fruit is assessed visually, there are no sharp deviations from the varietal characteristics.

In the sensory evaluation, the fruits of the five garden bean varieties were evaluated for the characteristics relevant to their suitability for fresh consumption (Table 3).

Table 3. Average sensory evaluations of garden bean cultivars

cultivar	texture	lyco	consistency	Preferences of consumers
Capitano	juicy	no	medium delicate	yes
Gina	Medium juicy	no	firm fruit	yes
Ebro	Medium juicy	no	firm fruit	yes
Bergold	Less juicy	no	firm fruit	no
Playa	Medium juicy	no	firm fruit	yes

Reference values for sensory qualities of vegetable crops are not given in the scientific literature. Currently, there is also no unified standard for evaluation (Paschova, 2021). Therefore, the sensory studies conducted are mainly based on the subjective opinion of evaluating consumers. Considering the combination of attractive bean appearance, texture and consistency, the most preferred varieties are Capitano and Gina. Other varieties are less favored, with the Bergold variety being the least desirable.

CONCLUSIONS

Higher yields were obtained from plants sown in the beginning of May. The difference with the yield from the second sowing date varied between 11 and 14%, with small differences between cultivars. The same plants also yielded 57% to 22% higher than the first harvest.
The results of the economic productivity of the plants and the morphological characteristics of the fruit give us reason to believe that the cultivars Capitano, Gina, Ebro and Playa can be recommended for cultivation with a view to enriching the cultivar range with new varieties of garden beans.

REFERENCES

Alemu, Y., Alamirew, S., & Dessalegn, L. (2017). Correlation and path analysis of green pod yield and its components in snap bean (Phaseolus Vulgaris L.) genotypes. International Journal of Research, 30.

Cholakov, D., 2009. Vegetable Production, Academic Publishing House of Agricultural University – Plovdiv, pp. 150-158. (in Bulgarien)

De Ron, A.M.; González, A.M.; Rodiño, A.P.; Santalla, M.; Godoy, L. and Papa, R. (2016). History of the common bean crop: its evolution beyond its areas of origin and domestication. Arbor, 192 (779): a317. doi: http://dx.doi.org/10.3989/arbor.2016.779n3007

http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567 FAOSTAT | © FAO Statistics Division 2010 | 30 August 2010 .

Kenneth, V. (2012). Evaluation of six fresh green bean varieties for pod quality and yield. *Gladstone Road Agricultural Centre Crop Research Report*, (9), 3-7.

Mihov, Kr., Panayotov, N., Filippov, St., Babrikov, T., Kostadinov, K., Haytova, D. 2014. Manual of Vegetable Production with Seed Production, Academic Publishing House of Agricultural University – Plovdiv (in Bulgarien)

Ministry of Agriculture, Department of Agro Statistics - Survey "Vegetable Production - Harvest `2022 (in Bulgarien)

Mirzaienasab, M., & Mojaddam, M. (2014). The effect of planting date on yield and yield components of two red bean cultivars in azna weather conditions. *Indian Journal of Fundamental and Applied Life Sciences*, *4*(3), 417-422.

Pandey Y.R., Gautam D.M., Thapa R.B., M.D. Sharma, K.P. Paudyal (2012) Response of Pole Type French Bean (*Phaseolus vulgaris* L.) Genotypes to Sowing Dates in the Mid Hills of Western Nepal, Nepal Journal of Science and Technology Vol. 13, No. 2 (2012) 15-20

Pashova S. , 2021. Quality of fresh fruits and vegetables, Journal of Mountain Agriculture on the Balkans, 2021, 24 (4), 378-401(in Bulgarien)

Rao, S. A. (2022): Impact of planting dates on yield and pod quality traits of snap bean under short-temperate season climates. International Journal of Horticultural Science 28: 57-63. https://doi.org/10.31421/ijhs/28/2022/11054

Shaban N.2014. Vegetable production, Publishing house at LTU, Sofia (in Bulgarien)

Sharma, A., Sharma, G. D., Singh, Y., Sharma, M., Katoch, V., & Sharma, K. C. (2013). Optimum sowing dates and varieties for seed productivity of pole Frenchbean (Phaseolus vulgaris L.) under north western Himalayas. African Journal of Agricultural Research, 8(48), 6196-6201.

Sparvoli, F., Bollini, R., & Cominelli, E. (2015). Nutritional value. *Grain legumes*, 291-325.

Topliceanu, D., Lulușa, V., Velicevici Giancarla, C. A., Ciulca, S., & Madoșă, E. (2018). Study of the adaptability of some bean (Phaseolus vulgaris L.) varieties to the conditions in western Romania. *JOURNAL of Horticulture, Forestry and Biotechnology*, *22*(4), 119-125.

MONITORING RESULTS FOR *SCAPHOIDEUS TITANUS* BALL (HEMIPTERA: CICADELLIDAE) IN GRAPE-GROWING REGION OF RAHOVEC IN KOSOVO

Gazmend GJİNOVCİ[1,2], Bekri XHEMALİ[2], Adnand RAMADHİ[1], Betim BRESİLLA[2,3] and Shpend SHAHİNİ[1]*

[1]*Department of Plant Protection, Agricultural University of Tirana, Tirana, Albania*
[2]*Laboratory of Plant Protection, Kosovo Institute of Agriculture, Pejë, Kosovo*
[3] *Department of Soil Science and Agrochemistry, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary*

**Corresponding author: gazmendgjinovci@hotmail.com*

ABSTRACT

The American grapevine leafhopper is a primary vector of the *Flavescence dorée* phytoplasma, posing a significant threat to vineyards worldwide. After the first record of leafhopper in the Kosovo vineyards in 2022, a comprehensive monitoring study was conducted during the year 2023 in order to assess the presence of first nymph and adult population dynamics of *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) in the grape-growing region of Rahovec, Kosovo. The monitoring program was carried out in four localities within the Rahovec region. Mainly wine varieties were the dominant population in all inspected vineyards. In order to study the first appearance of the nymph, the first and oldest leaves of the grapevine were inspected from the middle of May to the end of June 2023. While the adults were investigated and monitored from late June to early October 2023. Yellow sticky traps method was used across multiple vineyard sites throughout the growing season. Results indicated fluctuating population levels, with peak activity observed in mid-summer. The data collected provides essential insights for local viticulturists and can aid in developing targeted control measures to mitigate the impact of this pest and safeguard the viticulture industry in Rahovec.

Key words: *Scaphoideus titanus*, monitoring, grapevine, Kosovo

INTRODUCTION

The leafhopper *Scaphoideus titanus* Ball (Hemiptera, Cicadellidae) is a phloem-feeding Nearctic leafhopper originated from northeastern America, then it was first reported in Europe in the late 1950s (Bonfils & Schvester 1960). After its initial introduction in Europe, it spread from southwestern France to Italy, later to the Balkan countries and subsequently to Spain and Portugal (EFSA, 2020). The North American grapevine leafhopper years earlier was also identified in neighboring countries of Kosovo, in Serbia was first reported in 2004 (Magud and Toševski 2004) and in Montenegro was found in August 2008 (Radonjić et al., 2008). Recently, *Scaphoideus titanus* Ball has also been reported in Kosovo (Gjinovci et al., 2022). *Scaphoideus titanus* Ball is primarily ampelophagous, feeds on the lower surface of the leaves and completing its life cycle on *Vitis* species, including both European grapevines (*Vitis vinifera* L.) and American grapevines. In Europe, it has predominantly been reported on *Vitis vinifera*, although it is also known to thrive in abandoned vineyards and on wild grapevines (Rossi et al., 2019). However, it has recently been reported on other plant species as well (Alma et al. 2019). The leafhopper *Scaphoideus titanus* Ball serves as the main vector for *'Flavescence dorée'* (FD) phytoplasmas (Ripamonti et al., 2022). *'Flavescence dorée'* (FD) is an obligate bacterial pathogen that primarily reside in the phloem tissue of plants. When sap-sucking insects, such as *Scaphoideus titanus*, feed on infected plants, they can inadvertently acquire these pathogens and subsequently transmit them to healthy plants. Recent studies revealed that nymphs can acquire *'Flavescence dorée'* (FD) phytoplasmas by feeding on infected plants. After a latency period of 4–5 weeks, during which they mature into adults, they become vectors capable of transmitting the phytoplasmas to other plants (Belli et al., 2010; Ottati et al., 2020).

MATERİALS AND METHODS

The aim of this study was to detect the first appearance of the nymph emergence that was carried out by visually examining the underside of the oldest grapevine leaves from mid-May to late June 2023. Furthermore, adults were monitored from late June to early October 2023 using yellow sticky traps (Insect trap Aria® 10×25 cm) placed across different vineyard sites throughout the growing season. The monitoring survey was carried out during 2023 across four localities within the Rahovec region (Rahovec, Deshkidov, Hamovc and Opterush) (Fig. 1), where the dominant grapevine variety was the black variety Vranac.

Figure 1. *Map of Kosovo with particular focus in the region of Rahovec (Source: Wikipedia).*

To detect the presence of nymphs, three parcels from each locality were inspected. Nymphs collected from each surveyed locality were brought to the lab for morphological identification using a stereomicroscope (Olympus SZX16 Tokyo, Japan). In addition, adults were monitored weekly by directly counting individuals from grapevine plants using yellow sticky traps. The collected adults were preserved in 75% ethanol for later identification. The identification of adult specimens was conducted using the EFSA Pest Survey Card (2020) as the primary identification key. This card provides a systematic approach for accurately identifying nymph and adult specimens based on established morphological and diagnostic criteria.

RESULTS AND DİSCUSSİONS

During the 2023 survey, the initial inspection revealed the presence of both nymphs and adults of *Scaphoideus titanus* in four surveyed localities (Rahovec, Deshkidov, Hamovc and Opterush). Following a detailed visual inspection, the initial presence of first nymphal instars was documented at the beginning of June 2023 in the locality of Hamovc, within the Rahovec region. These nymphal instars were predominantly observed on the lower surface of the oldest grapevine leaves, which were situated closest to the bark of the grapevine plants (Fig. 2). During visual inspections, larval skins were also observed on the lower surface of grapevine leaves. According to Aldini et al. (2003), examining the lower surface of the leaves is more effective for identifying the immature stages of *Scaphoideus titanus*.

Figure 2. *Scaphoideus titanus nymph: left – first instar; right – fifth instar.*

Based on the observed number of nymphs and the sporadic presence of larval skins, the population density of *Scaphoideus titanus* was classified as low. Specifically, there were 3-5 nymphs detected on grapevine leaves per vineyard. Through continuous monitoring over the subsequent months, the initial presence of adult specimens was recorded at the beginning of July 2023 in the locality of Hamovc, within the Rahovec region (Fig. 3). The population density showed a consistent low level over two decades of July 2023, with adult individuals captured fluctuating between 1 and 10 specimens. In contrast, during the last decade of July 2023 and the first decade of August 2023, the capture increased to between 10 and 40 specimens (Figure 4). This observation suggests a rapid dispersal and establishment of *S. titanus* across all inspected sites within a short timeframe. The findings underscore the need for continuous monitoring and implementation of management strategies to mitigate the spread and potential impact of this pest on local viticulture.

Figure 3. *S. titanus adult*

Figure 4. *Collecting adults using yellow sticky traps*

CONCLUSIONS

The data indicates that *Scaphoideus titanus* Ball is an established pest in the Rahovec region, the largest grapevine-growing area in Kosovo. Although the population density of this leafhopper was categorized as low in 2022, consistent with previous years, its recurrent detection in the Hamovc locality signifies its adaptation to the agroecological conditions of this viticultural area. Consequently, there exists a tangible risk of *S. titanus* progressively invading the entire region. The established presence and potential dissemination of *S. titanus* could pose a significant threat due to the possible occurrence of *Flavescence dorée* (FD) phytoplasmas.

REFERENCES

Aldini, R. N., Ciampitti, M., & Cravedi, P. 2003. Monitoring the leafhopper *Scaphoideus titanus* Ball and the planthopper Hyalesthes obsoletus Signoret in Northern Italy. *IOBC WPRS BULLETIN*, *26*(8), 233-236.

Alma, A., Lessio, F., & Nickel, H. 2019. Insects as phytoplasma vectors: ecological and epidemiological aspects. In B. Assunta, G. W. Phyllis, P. R. Govind, & M. Nicola (Eds.), Phytoplasmas: Plant Pathogenic Bacteria-II (pp. 1–25). Singapore: Springer. https://doi.org/10.1007/978-981-13-2832-9_1

Bonfils, J., & Schvester, D. 1960. Les cicadelles (Homoptera: Auchenorrhyncha) dans leurs rapports avec la vigne dans le Sud-Ouest de la France. *Annales des Épiphyties*, 11(3), 325–336.

European Food Safety Authority (EFSA), Tramontini, S., Delbianco, A., & Vos, S. 2020. Pest survey card on *Flavescence dorée* phytoplasma and its vector *Scaphoideus titanus*. *EFSA Supporting Publications*, *17*(8), 1909E.

Gjinovci, G., Xhemali, B., Bresilla, B., Shahini, S., Morina, N., & Bajrami, T. 2022. Preliminary investigations of the main *Flavescence dorée* vector *Scaphoideus titanus* and its first record in Kosovo. *Travaux du Muséum National d'Histoire Naturelle "Grigore Antipa"*, *65*(2), 81-86. https://doi.org/10.3897/travaux.63.e98562

Magud, B., & Toševski, I. 2004. *Scaphoideus titanus* Ball. (Homoptera, Cicadellidae): a new pest in Serbia.

Radonjić, S., Hrnčić, S., Krstić, O., Toševski, I., Jović, J. 2012. Presence and distribution of *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) in the vineyards of Montenegro. In: Proceedings of the International Symposium on Current Trends in Plant Protection, Institute for Plant Protection and Environment, Belgrade, Serbia, 25–28th September 2012, pp. 506–510.

Ripamonti, M., Galetto, L., Maron, F., Marzachì, C., & Bosco, D. 2022. *Scaphoideus titanus* fitness on grapevine varieties with different susceptibility to *Flavescence dorée* phytoplasma. *Journal of Applied Entomology*, *146*(10), 1260-1271.

Rossi, M., Pegoraro, M., Ripamonti, M., Abbà, S., Beal, D., Giraudo, A., ... & Marzachì, C. 2019. Genetic diversity of Flavescence dorée phytoplasmas at the vineyard scale. *Applied and Environmental Microbiology*, *85*(10), e03123-18.

INFLUENCE OF DRIP IRRIGATION AND FERTIGATION ON PHYSICAL CHARACTERISTICS OF WHITE STRAWBERRY FRUITS GROWN IN BULGARIA

Elena GRANCHAROVA[1], Petya IVANOVA[2], Blagoj ELENOV[1], Stoil ZHELYAZKOV[2]

[1] Institute of soil science, agrotechnologies and plant protection „Nikola Poushkarov", Agricultural Academy, Bulgaria
[2] Institute of Food Preservation and Quality, Agricultural Academy, Bulgaria
Corresponding author e-mail: eveha@abv.bg

ABSTRACT

The aim of this paper is to present the effects of the applied regimes of fertilization and irrigation on the physical characteristics of white strawberry fruits. A two factors experiment was conducted during 2024 in unheated greenhouse in the Chelopechene experimental field, Sofia, Bulgaria with drip irrigated and fertigated strawberry variety (*Fragaria x Ananassa „Snow White"*). The irrigation and the fertilization factors were applied in two rates: I1 - 75% (ETc) I2 - 50% (ETc), F1: optimal fertilization $N_{8.09}P_{12.76}K_{15.62}$; F2 – suboptimal fertilization - 75% (F1). Five treatments were tested: control: I0F0:100% (ETc) without fertigation; I1F1; I1F2; I2F1; I2F2. The highest percent (26.02%) "Extra" Class fruits were obtained from I1F1 treatment and the highest average fruit weight in "Extra" Class was obtained from I1F2 treatment – 7.91 g.
It was found that the method of cultivation and irrigation affects the instrumentally measured physical parameters of fruit firmness and color ($p<0.05$). The applied agricultural techniques influenced the measured parameters of firmness and adhesion only for the fruits grown with 75% irrigation and 75% fertilization, as well as for the adhesion parameter in fruits grown with 50% irrigation and 75% fertilization. For the remaining fruits studied in the planned experiment variants, the method of cultivation did not affect the values of the mentioned parameters ($p>0.05$).
The color parameters—brightness, yellow hue, color saturation, and color difference—did not influence the quality of the fruits across all analyzed cultivation variants ($p>0.05$). The best qualitative and quantitative color parameters, including measured red hue, saturation, and shade of color, were observed in the fruits of the control group with 100% irrigation, followed by the fruits grown with 50% irrigation and 75% fertilization. For the other fruits from the studied cultivation variants, no effect on the measured color parameter values was found after statistical data processing ($p>0.05$).

Keywords: Irrigation, Fertigation, White strawberries, Color, Firmness, Physical parameters,

INTRODUCTION

Fragaria Ananassa berries are an important source of vitamins, minerals and fibers and widely consumed fruit. They are produced by open-field cultivation, by greenhouse cultivation (Ivanova et al., 2024), by hydroponic cultivation (Balan et al., 2024), under mulch (Taparauskiene and Miseckaite. 2014) and other cultivation practices, technologies and mechanization techniques. At the same time strawberry is one of the most drought sensitive plants. Soil moisture deficit has a negative impact on strawberry growth, development, yield, berry size, persistency in winter time (Taparauskiene, 2006).

The physical parameters of strawberry fruits - diameter (Akhijahcmi and Khodaei, 2011), weight (Birania et al., 2022) can range vary widely based on cultivar and environmental conditions.

Strawberries with white fruits have long history of cultivation no shorter then red varieties. White strawberry has cultivated for hundreds of years in Chile and has grown in two botanical forms - wild *Fragaria chiloensis ssp. chiloensis f. patagonica* and cultivated *Fragaria chiloensis ssp. chiloensis f. chiloensis* (Grez et al., 2020). It was brought to Europe in the 18th century. „Snow White" cultivar has been selected in 2010 out from *Fragaria x ananassa "Weisse Ananas"* and *Fragaria chiloensis f. Chiloensis* (Olbricht et al., 2013). White strawberries are distinguished by their white or pale pink flesh and unique pineapple-like flavor.

Research on white strawberries is still limited and with current studies focusing on their genetic diversity (Lu et al., 2021), cultivation requirements (Whitaker, 2023), potential health benefits (Lin et al., 2018), sugar content (Seki et al., 2021), chemical composition and biological activities (Fierascu et al., 2020). This indicates that there is substantial scope for further research in this area.

This research investigates the effects of drip irrigation and fertigation on white strawberries' physical characteristics.

MATERIAL AND METHOD

✓ Experimental layout

The second year two-factor experiment was conducted on drip irrigated strawberry plants in a tunnel greenhouse in 2024 in the Chelopechene experimental field (latitude 42°44′22.8″N, longitude 23°28′3.7″E and altitude 550 m above sea level) of the Institute of soil science, agrotechnologies and plant protection "Nikola Poushkarov" in Sofia, Bulgaria. Sofia field falls into temperate continental climate subzone. The greenhouse was unheated with area 420 m^2 (7.9 m x 53 m) covered with a five-layer UV+EVA+IR+AD+dif -150 μm polyethylene film. The soil could be defined as moderate to strong water-permeable with an average filtration capacity. The soil was *Chromic Luvisol* with bulk density 1.47 g cm^{-3}, field capacity 22% and wilting point 10% for 0-50 cm layer. To further reduce water losses, mulching with silver-black UV polyethylene mulch with a thickness of 30 μm was applied.

The object of the study was white strawberry cultivar (*Fragaria × ananassa "Snow White"*). The experimental treatments were arranged according to the method of long plots with three replications. Each plot has 23.2 m^2 area and consisted of twin rows of strawberries. Healthy bare-root frigo plants were planted in scheme of 90 + 30/30 cm on 22 March 2023. According to the white strawberries cultivation technology in each of the experimental plots were provided the appropriate amount of red fruit plants (4: 1 ratio) to ensure better pollination. The irrigation factor was applied in two rates: I1 – deficit irrigation - 75% (ETc); I2 - deficit irrigation - 50% (ETc). The fertilization factor was applied in two rates: F1: optimal fertilization $N_{8.09}P_{12.76}K_{15.62}$; F2 – suboptimal fertilization - 75% (F1) - $N_{6.07}P_{9.57}K_{11.94}$. Optimal fertilization was developed according to Haifa nutrition recommendations (Haifa Group, 2021) as follows: Haifa MAP- 25-45 kg ha^{-1} , Multi K – 80 kg ha^{-1} , Haifa MKP - 25 kg ha^{-1} , Haifa Cal - 30 kg ha^{-1} , Maguisal - 10 kg ha^{-1} , Poly-feed – 25-40 kg ha^{-1}. Five treatments were tested: control treatment I0F0: 100% (ETc) - full irrigation and without fertigation; I1F1; I1F2; I2F1; I2F2. Irrigation was applied trough drip system include NMC Junior controller for precise irrigation rate application, FertiKit Nutrigation system for precise fertigation rate application, pressure-compensated pipelines UniRam AS with 14.6 mm inside diameter, 1.2 mm wall thickness, built-in trough 20 cm drippers and flow rate 1.6 l h^{-1}.

The microclimate data (air temperature, relative humidity, solar radiation, sunshine duration and wind speed) in the greenhouse was measured at every 30 min using an automatic meteorological station located in the center of experimental area and recorded in data logger (HOBO USB Micro Data Logger, USA). FAO Penman-Monteith Equation (Allen et al., 2006a) was used for determining daily reference evapotranspiration and irrigation scheduling. Crop coefficient was 0.30, 0.80 and 0.70 respectively in initial, middle and end growing stage (Allen et al., 2006b). Fruit mass was determined through weighing with a electronic precision balance Vedia FR-H (±0.01 g). Fruit width and length was measured with a digital calliper (±0.01 mm). All the observations were carried out of 5 consecutive plants in three replications. Marketing standard strawberry classification was made according to Commission implementing regulation (EU) No 543/2011. The minimum fruit diameter for "Extra" Class - 25 mm and for Class I and Class II - 18 mm.

- ✓ **Methods**

Colour – Instrumental with colorimeter (PCE-CSM 5 portable colorimeter – measurement geometry 8°/d, Ø 8 mm, Light source D65)

The indicators are reported according to the CIE Lab system.

During the measurement 3 colour coordinates were taken: L, a и b;

- ➢ **L** – colour brightness (L=0 - black, L=100 – white);
- ➢ **a** – the positive values of the indicator characterize the amount of red color, whereas the negative ones characterizes the green color;
- ➢ **b** – positive valuescharacterize the yellow colour, whereas negative values characterize the blue color (ASTM D2244-16).
- ➢ **Saturation** – saturation characterizes the degree of dilution of the color tone with white color (white color saturation is 0).

$$C=\sqrt{(a^2+b^2)} \qquad (1)$$

- ➢ **Colour tone** It determines the position of the color in the respective quadrant and represents the ratio a/b.

A lower value of color tone implies a smaller change in color and vice versa.

- ➢ **Colour differences** between individual samples are determined by the values ΔL, Δa and Δb, and ΔE is a summary indicator of the final colour.

Colour differences ΔE is determined by the formula:

$$\Delta E=\sqrt{(\Delta L^2+\Delta a^2+\Delta b^2)}, \qquad (2)$$

where:

$\Delta L = Li – L0$

$\Delta a = ai – a0$

$\Delta b = bi – b0$

“0” – sample-variant control

“i” – sample-variant different growing

Saturation (C), colour tone (a/b) and colour differences ΔE are the parameters that characterize the color quality in the so-called physiological visual system.

Five measurements were performed on each sample. The colour coordinates of each sample represent arithmetic mean values of the measured coordinates.

The texture was analyzed using a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) in penetration mode. A cylindrical probe with a diameter of 5 mm was used for this purpose. The deformation speed was 1 mm/s, and the deformation was 5 mm. Ten replications were conducted for each cultivation variant of white strawberries. This test determined the firmness, adhesiveness, and Young's modulus (stiffness) of the white strawberry fruits. The rupture curves indicate the values of Young's modulus as the slope of the first linear section, as well as the deformation and stress at yield and at the rupture point.

The obtained data were statistically analyzed using ANOVA software, as well as Microsoft Excel and STATISTICA 8.0. Duncan's Multiple Range tests at a significance level ($p<0.05$) to measure specific differences between pairs of means was used.

RESULTS AND DISCUSSION

In 2024, an analysis of white strawberry fruits was conducted, presenting data on the impact of irrigation and fertigation on the physical characteristics of the fruits on the day of their harvest. The weight and diameter of the fruits were measured for all cultivation variants in the experimental greenhouse at the Institute of soil science, agrotechnologies and plant protection „Nikola Poushkarov", Sofia, in accordance with the quality requirements for fruits according to the general market standard for strawberry quality.

All fruits from the studied variants were healthy; intact; fresh; unwashed; practically free from visible foreign matter; undamaged by pests; free from off-flavors and odors. The fruits had a satisfactory level of ripeness and were delivered in refrigerated bags to the laboratory of the Institute of Food Preservation and Quality - Plovdiv, for conducting physical analyses, including firmness and instrumentally measured color.

The results (Table 1) showed that the highest percent (26.02%) "Extra" Class fruits were obtained from I1F1 treatment while the lowest percent (5.13%) "Extra" Class fruits were obtained from I2F2 treatment. The strawberries have a glossy appearance, with no traces of soil and no defects. Up to 5% of the number or weight of the strawberries may not meet the requirements for this class but must meet the requirements for Class I, or in exceptional cases, correspond to the permissible deviations for Class I. Of these 5%, no more than 2% may be fruits with damage.Expected, with the decrease of irrigation and fertigation rate, the fruit diameter also deceases. Reduction of the fruit diameter between the highest (I1F1) and the lowest (I2F2) values was 21.24%. Results of Duncan's Multiple Range test show that there was no significant difference between the varietal means of control treatment I0F0 and I1F1 and between the varietal means of I0F0 and I1F2. Also, there was no significant difference between the varietal means of I2F1 and I1F2. Between other pairs differences were significant at $p=0.05$.

Table 1. Marketing standard strawberry classification percentage by treatments

Treatment	"Extra" Class (%)	Class I and Class II (%)	Class III (%)
I0F0	24.15^{a}	64.76^{a}	11.09^{abc}
I1F1	26.02^{ab}	60.80^{a}	13.18^{abcd}
I1F2	21.61^{abc}	65.08^{a}	13.31^{bcd}
I2F1	14.94^{c}	64.31^{a}	20.74^{bcd}
I2F2	5.13	51.86	43.01

Values with same lowercase letter for the same parameter were not statistically different.
NS=not significant

The results (Table 1) showed that the highest percent (65.08%) Class I and Class II fruits were obtained from I1F2 treatment while the lowest percent (51.86%) were obtained from I2F2 treatment. They are of good quality, with minor defects in shape, slight pressure marks, and small white spots on the surface, free from soil and other defects that do not worsen the overall appearance, quality, or shelf life of the strawberries. Up to 10% of the number or weight of the strawberries may not meet the requirements for this class but must meet the requirements for Class II, or in exceptional cases, correspond to the permissible deviations for Class II. Of these

10%, no more than 2% may be fruits with damage. Results of Duncan's Multiple Range test show that only between I2F2 and other treatments differences were significant at p=0.05.

The results (Table 1) showed that the highest percent (43.01%) Class III fruits were obtained from I2F2 treatment while the lowest percent (11.09%) were obtained from I0F0 treatment. Results of Duncan's Multiple Range test show that there was no significant difference between the varietal means of I0F0 and I1F1. Also, there was no significant difference between the varietal means of I0F0, I1F1, I1F2 and I2F1. Between I2F2 and other treatments differences were significant at p=0.05.

With the decrease of irrigation rate and fertigation rate, the mean fruit diameter also decreases. Similar results have been reported by Bibi et al. (2016) for red variety.

The results (Table 2) showed that the highest average fruit weight in "Extra" Class was obtained from I1F2 treatment – 7.91 g. The highest average fruit weight in Class I and Class II was obtained from I1F1 treatment – 4.49 g. The highest average fruit weight in Class III was obtained from I1F2 treatment – 2.19 g. Expected, with the decrease of irrigation and fertigation rate, the fruit weight also deceases. Similar results have been reported by Kang et al. (2018), Kachwaya et al. (2015) for red variety.

Table 2.Average fruit weight according Marketing standard strawberry classification by treatments

Treatment	"Extra" Class Avg. fruit weight (g)	Class I and Class II Avg. fruit weight (g)	Class III Avg. fruit weight (g)
I0F0	7.51	4.45	2.00
I1F1	7.87	4.49	2.12
I1F2	7.91	4.46	2.19
I2F1	7.57	4.25	1.99
I2F2	6.98	3.85	1.91

The indicators of interest to consumers are visual appearance, texture/firmness, color, nutritional value, and food safety. The data from the instrumentally measured color parameters are presented in Figure 1.

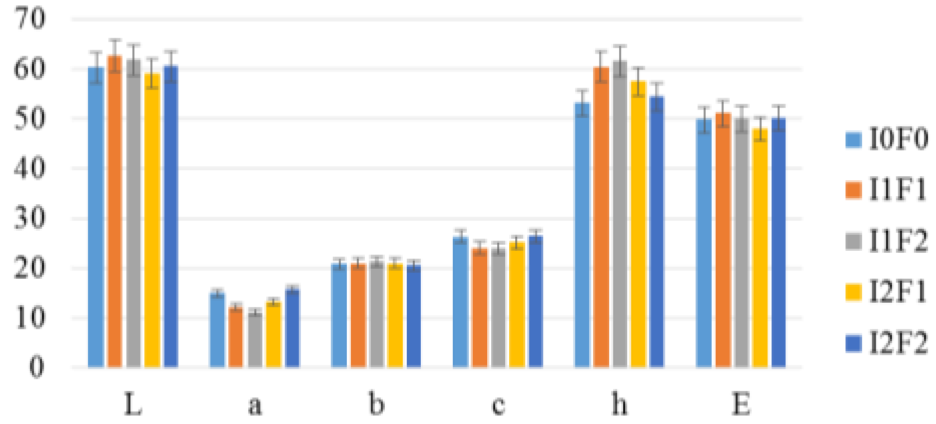


Figure 1. Color parameters of white strawberry fruits by treatments

The data from Figure 1 show that the color brightness values for all fruits from the cultivation variants do not differ statistically, and the cultivation method does not affect this color parameter ($p>0.05$) (Figure 1).

A significant difference was noted in the measured red hue of the examined fruits. Statistically distinct differences were observed in the fruits grown with 75% irrigation and 75% fertilization, with a value of 11.12, which is the lowest compared to the results reported for the other fruit variants. The cultivation method affects the red hue ($p<0.05$) (Figure 1). The highest statistically non-different red color values of 15 were recorded for the control fruits with 100% irrigation and for fruits grown with 50% irrigation and 75% fertilization, followed by the other two cultivation variants with 50% irrigation and 100% fertilization and 75% irrigation and 100% fertilization. For these last-mentioned variants, the cultivation method does not influence the reported red color values ($p>0.05$) (Figure 1).

The yellow hue of the fruits from all examined cultivation variants dominates over the red hue on the day of harvest and is around 20. The cultivation method (irrigation and fertigation) does not influence the yellow color component ($p>0.05$) (Figure 1).

Color saturation characterizes the degree of dilution of the color hue with white, with the recorded values being closer to the saturation of the white color above 23. The cultivation method (irrigation and fertigation) on the harvest day does not affect the measured color saturation ($p>0.05$) (Figure 1).

The color tone of fruits from variants with 100% irrigation and with 50% irrigation and 75% fertilization had the best values, statistically non-different from each other (above 53) ($p>0.05$) (Figure 1). Greater color changes were observed in fruits grown with 75% irrigation and 100% fertilization and with 75% irrigation and 75% fertilization (above 60), statistically non-different from each other ($p>0.05$) (Figure 1). The cultivation method (irrigation and fertigation) influences the measured color tone of the above-mentioned variants ($p<0.05$) (Figure 1).

The calculated color difference of the fruits for all studied variants showed that the irrigation and fertigation methods do not affect the color difference ($p>0.05$) (Figure 1).

Texture/firmness was assessed using destructive methods cited in the Materials and Methods section. Fresh strawberry fruits are highly susceptible to damage from mechanical handling. To prevent macro damage from external forces and to predict the evolution of damage within the internal tissues, the mechanics of texture damage to strawberry fruits and their tissues are characterized through load and unload tests at different compression speeds. The measured texture parameters include firmness, elasticity, and adhesiveness (stickiness).

The texture parameters of the studied cultivation variants for white strawberries are presented in Figures 2, 3, and 4.

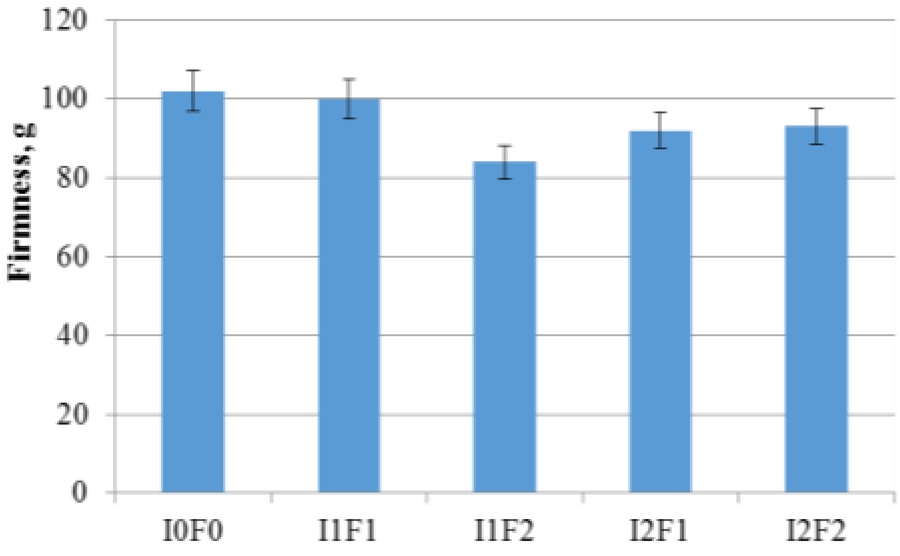


Figure 2. Firmness of white strawberry fruits by treatments

The white strawberries from the control group with 100% irrigation and those with 75% irrigation and 100% fertilization have the highest values for measured firmness, 102.1 g and 100.2 g respectively, followed by fruits grown with 50% irrigation and 100% fertilization, and 75% fertilization and 50% irrigation, which have statistically non-different values from each other ($p>0.05$). The white strawberries from the cultivation variant with equal percentages of 75% irrigation and 75% fertilization have statistically different firmness values (84 g). The cultivation method affects the firmness values of the fruits ($p<0.05$) (Figure 2).

The adhesiveness (stickiness) indicator for the examined fruits showed that the stickiness of fruits grown with 50% irrigation and 75% fertilization is the highest (-12.44 g•sec). Fruits from the control group with 100% irrigation, 75% irrigation and 100% fertilization, and 50% irrigation and 100% fertilization have statistically non-different stickiness values ($p>0.05$) (Figure 3).

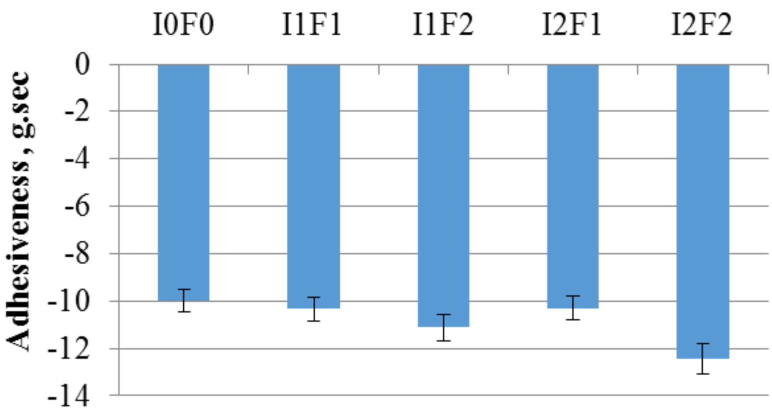


Figure 3. Adhesion (stickiness) of white strawberry fruits by treatments

The elasticity of the fruits depending on the cultivation method shows no statistically significant differences (p>0.05). All examined fruits exhibit the same degree of deformation under the applied force for structural breakdown.

The cultivation method of white strawberry fruits does not affect the elasticity parameter on the day of harvest (Figure 4).

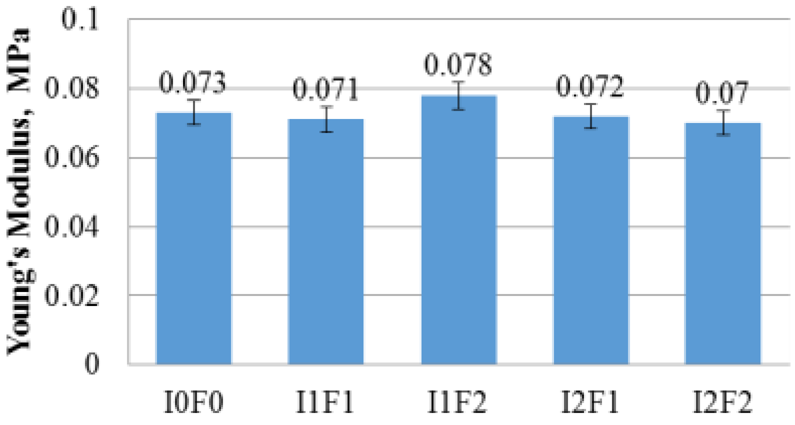


Figure 4. Elasticity of white strawberry fruits by treatments

The applied agronomic techniques affect the measured firmness and adhesion parameters only for fruits grown with 75% irrigation and 75% fertilization, as well as for the adhesion parameter of fruits grown with 50% irrigation and 75% fertilization.

No linear correlation was found between the instrumentally measured firmness parameters and the seniority assessed consistency parameter.

For the remaining examined fruits from the planned experimental variants, the cultivation method does not influence the values of the cited parameters (p>0.05).

CONCLUSIONS

The aim of this paper is to present the effects of the applied regimes of fertilization and irrigation on the physical characteristics of white strawberry *Fragaria × ananassa "Snow White"*. The highest percent (26.02%) "Extra" Class fruits were obtained from I1F1 treatment while the lowest percent (5.13%) "Extra" Class fruits were obtained from I2F2 treatment. The highest percent (65.08%) Class I and Class II fruits were obtained from I1F2 treatment while the lowest percent (51.86%) were obtained from I2F2 treatment. that the highest percent (43.01%) Class III fruits were obtained from I2F2 treatment while the lowest percent (11.09%) were obtained from I0F0 treatment. The results showed that the highest average fruit weight in "Extra" Class was obtained from I1F2 treatment – 7.91 g. The highest average fruit weight in Class I and Class II was obtained from I1F1 treatment – 4.49 g. The highest average fruit weight in Class III was obtained from I1F2 treatment – 2.19 g. The cultivation method does not affect color brightness, red hue, the yellow color, color saturation and color difference but affects the color tone. The cultivation method affects the firmness values of the fruits

The results show that water and nutrient regimes are the main factors, which affect yield, berry size and others features of strawberry development.

ACKNOWLEDGEMENTS

This research work was carried out with the financial support of the BULGARIAN NATIONAL SCIENCE FUND „Competition for financial support for basic research projects – 2022", project title "Impact of the soil and weather parameters on the irrigation management and elaboration of biological means to control phytopathogens to enhance strawberry fruit quality" (grant No КП-06-Н66/4).

The authors also want to thank *Haifa South East Europe* for providing whole range of water soluble fertilizers for conducting the experiments.

REFERENCES

Akhijahcmi, H.S., J. Khodaei. 2011. Some physical properties of strawberry (*Kurdistan variety*). World Applied Sciences Journal 13(2):206-212

Allen, R. G., L. S. Pereira, D. Raes, M. Smith. 2006a. Crop evapotranspiration (guidelines for computing crop water requirements). FAO Irrigation and drainage paper 56. (Food and Agriculture Organization of the United Nations, Rome), pp.24.

Allen, R. G., L. S. Pereira, D. Raes, M. Smith. 2006b. Crop evapotranspiration (guidelines for computing crop water requirements). FAO Irrigation and drainage paper 56. (Food and Agriculture Organization of the United Nations, Rome), pp.138.

Balan, H., A. Melnychenko, V. Sergiyenko, Y. Enakiev, D. Domuschi. 2024. Improvement of technology in the cultivation of strawberries hybrid Soraya F1 during a vegetative trial. BIO Web of Conferences 122, 01024 EE&AE-2024, 1-13. https://doi.org/10.1051/bioconf/202412201024

Bibi, Sh., Sh. M. Khan, A. Rehman, I. Ur-rahman, F. Ijaz, Sohail, A.Afzal, R. Khan. 2016. The effect of potassium on growth and yield of strawberry (Fragaria ananassa (duchesne ex weston) duchesne ex rozier). Pakistan Journal of Botany, 48(4): 1407-1413.

Birania, S., A. K. Attkan, S. Kumar, N. Kumar, V. K. Singh, 2022. Mass modeling of strawberry (Fragaria × Ananasa) based on selected physical attributes. Journal of Food Process Engineering, 45(5), e14023. https://doi.org/10.1111/jfpe.14023

Grez, J., E. Contreras, S. Sánchez, J.A. Alcalde, M. Gambardella. 2020. Floral induction and dormancy behaviour in "*Chilean white strawberry*" *(Fragaria chiloensis (L.) Mill. subsp. chiloensis f. chiloensis)*. Scientia Horticulturae, 274, 109648. https://doi.org/10.1016/j.scienta.2020.109648

Haifa Group. (2021). https://www.haifa-group.com/crop-guide/vegetables/strawberry-fertilizer/crop-guide-strawberry-fertilizer-recommendations

Ivanova P., B. Elenov, E. Grancharova. 2024. Influence of irrigation and fertigation on sensory characteristics of fruits of white strawberry (*Fragaria x anannassa "Snow White"*) grown in Bulgaria. Scientific Papers. Series B, Horticulture, Vol. LXVIII, Issue 1, pp. 462-468.

Kachwaya, D., J. Chandel, G. Vikas, B. Khachi. 2015. Effect of fertigation on growth, yield, fruit quality and leaf nutrients content of strawberry (Fragaria × ananassa) cv Chandler. Indian Journal of Agricultural Sciences, 85 (10), 1319–23.

Kang, R., J. Niu, Zh. Chen, J. Zhang. 2018. Effects of different water-soluble fertilizers on yield and quality of strawberry under integrated application of water and feltilzer. Asian Agricultural Research, 10(12), 59-66. https://doi.org/10.19601/j.cnki.issn194-9903.2018.12.016

Lin, Y., L. Jiang, Q. Chen, Y. Li, Y. Zhang, Y. Luo, Y. Zhang, B. Sun, X. Wang, H. Tang. 2018. Comparative transcriptome profiling analysis of red- and white-fleshed strawberry (*Fragaria×ananassa*) provides new insight into the regulation of the anthocyanin pathway, Plant and Cell Physiology, 59 (9), 1844–1859. https://doi.org/10.1093/pcp/pcy098

Lu, J., Y. Zhang, X. Diao, K. Yu, X. Dai, P. Qu, M. J. Crabbe, T. Zhang, Q. Qiao. 2021. Evaluation of genetic diversity and population structure of Fragaria nilgerrensis using EST-SSR markers, Gene, 796–797, 145791. https://doi.org/10.1016/j.gene.2021.145791

Olbricht, K., U. Gerischer, A. Ludwig, D. Ulrich, B. Casas, A. Darbonne, S. Kraege, H. Obers, A. Vissers, P. Walpole, S. Walpole. 2013. "Snow White", a new strawberry cultivar with white fruits. Book of abstracts Growing strawberries in the next decade: intonation, the key of success? International Strawberry congress, Belgium. Retrieved July 1 2021, from https://iris.unipa.it/retrieve/handle/10447/99579/128625/Book%20of%20abstracts%20ISC2013.pdf

Taparauskiene, L. 2006. Influence of irrigation on strawberries "Senga Sengana." Sodininkystė ir daržininkystė. Scientific Articles 25, (4), 124

Taparauskiene, L.,O. Miseckaite. 2014. Effect of mulch on soil moisture depletion and strawberry yield in sub-humid area. Polish Journal of Environmental Studies, 23(2): 475-482

Whitaker, V. M., C. Dalid, L. F. Osorio, N. A. Peres, S. Verma, S. Lee, A. Plotto. 2023. Florida Pearl® 'FL 16.78-109' Pineberry. HortScience, 58(1), 143-146. https://doi.org/10.21273/HORTSCI16951-22

EFFECTS of MICROBIAL FERTILIZER on YIELD and QUALITY of CURLY LETTUCE GROWN in POTS

Ufuk Uçan[1*] Halil Demir[1] H. Kayhan Yalci[2]

[1] *Department of Horticulture, Faculty of Agriculture, University of Akdeniz, Antalya 07070, Turkey*
[2] *Material Scientist, Herasim Agricultural Products Compan., Kocaeli, Turkey*
**Corresponding author e-mail: ucanufuk@gmail.com*

ABSTRACT

Microbial fertilisers containing various microorganisms that are in a symbiotic relationship within each other and with plant roots have positive effects on plant growth, development and flowering. They can be used in conventional, organic and sustainable agriculture systems and reduce chemical fertilizer consumption. In this study conducted under greenhouse conditions, the effects of Herasim microbial fertiliser on yield and some quality characteristics of lettuce were investigated. Caipira (*Lactuca sativa* var. *crispa*) lettuce cultivar was used as plant material. In pot trials, 70% peat + 30% perlite mixture was used as a growing medium. A total of 9 different treatments were included in the research: Control (C), 100% Chemical Fertilisation (100% CF), 50% Chemical Fertilisation + Microbial Fertilisation (50% CF + MF), 75% Chemical Fertilisation + Microbial Fertilisation (75% CF + MF), 100% Chemical Fertilisation + Microbial Fertilisation (100% CF + MF), Immersion + Chemical Fertilisation (I + CF), Microbial Fertilisation alone (MF), 50% Chemical Fertilisation + Microbial Fertilisation + Foliar Microbial Fertilisation (50% CF + MF + FMF), 75% Chemical Fertilisation + Microbial Fertilisation + Foliar Microbial Fertilisation (75% CF + MF + FMF). Head height (cm), root collar diameter (mm), number of leaves (number/plant), leaf colour (L*, a* and b*), chlorophyll (SPAD), soluble solids (%SS), pH, total and marketable yield (g/plant) criteria were examined. The total and marketable yield results obtained from 100% CF + MF, I + CF, 75% CF + MF, 75% CF + MF + FMF and 50% CF + MF + FMF treatments were the highest and very similar to that of 100% CF treatment. In addition, similar or better results were obtained fort the same treatments in terms of lettuce growth, colour, chlorophyll and SS criteria.

Key words: Bacteria, biofertiliser, chlorophyll, colour, quality

INTRODUCTION

According to data from the Turkish Statistical Institute, our country's vegetable production in 2023 is 31.8 million tons. The most produced vegetable is tomato with 13.3 million tons, followed by watermelon with 3.2 million tons and onion with 2.6 million tons. The production of leafy vegetables, including lettuce, is more than 2 million tons. Lettuce production is 577 thousand tons (TUIK 2024). Lettuce, one of the annual cool-climate vegetables whose leaves are consumed (Aćamović-Đoković et al. 2011; Colonna et al. 2016) mostly as salads and processed vegetables, are of great importance for human nutrition due to their rich vitamins, minerals, and antioxidants. Raw lettuce contains more nutrients than processed lettuce (Aćamović-Đoković et al. 2011).

One of the most significant challenges facing vegetable cultivation in our country is the relatively low yield per unit area, which varies depending on the region and on the species being cultivated (Aksoy and Altındişli, 1998). Since the beginning of the 20th century, chemical fertilizers, hormones and pesticides have been used extensively to increase yields (Aksoy 1999). In today's intensive modern agriculture, the main objective is to achieve the maximum yield. However, the potential damage to the soil and the environment caused by chemical fertilisers is often overlooked, as highlighted by Parr et al. (1994). Chemicals lead to deterioration of soil structure and biological balance in the soil. Therefore, alternative organic inputs for agriculture are being investigated to regain the lost fertility and biological balance, and to grow crops that will bring benefit to environment and human health Biofertilizers or microbial fertilizers are among these alternative products with significant potential.

Microbial fertilizers are organic products that generally contain microorganisms obtained from plant roots or root zones (Chen 2006; Gupta and Sen 2013). In other words, microbial fertilizers are products that contain different types of microorganisms that can mobilize elements in the soil that cannot be used by plants (Muraleedharan et al. 2010). Cocktail mixtures known as Effective Microorganisms consisting of lactic acid bacteria, phototrophic bacteria and yeast (Goessler and Kuehenelt, 2002) are used in plant cultivation, are physiologically compatible with each other and can coexist by working in a symbiotic relationship. There are studies on the possibility that beneficial microorganisms can increase soil quality, plant growth and yield (Kengo and Hui-lian, 2000). In addition, yeasts produce hormones and enzymes that promote plant cell and root division. Different microorganisms in cocktail mixtures complement each other and establish a symbiotic relationship with plant roots in the soil.

Plants grow extremely well in soils where effective microorganisms live (Pei-Feng et al. 2014). Plant growth promoting bacteria (PGPR) such as rhizobacteria are widely used as biofertilizers for many crops as well as vegetables (Gravel et al. 2007). Such organisms increase the availability of nutrients to the plant by reducing ethylene levels in plants or increasing the production of plant growth regulators such as indole-3-acetic acid (IAA). Physiological mechanisms also change the morphology of roots, facilitating root growth and increasing nutrient uptake (Vessey 2003; Antoun and Prévost 2005; Richardson et al. 2009). Microorganisms are also known to be capable of developing defences against both biotic and abiotic stress factors (Pieterse et al. 2014; Prasanna et al. 2014; Triveni et al. 2015). Beneficial microorganisms not only suppress soil-borne pathogens but also increase the decomposition of organic materials and the availability of plant nutrients and important organic compounds to plants (Singh et al. 2003). They have significant positive effects on improving soil fertility, plant growth, flowering, fruit development and ripening (Lévai et al. 2006). They can be used in conventional, organic and integrated farming systems to reduce the consumption of chemical fertilizers and pesticides and minimize their negative effects on the ecosystem (Molla et al. 2012). Microorganisms activate nutrients (macro and micro elements) and support plant growth (Colla et al. 2010; Wang et al. 2022).

Leafy vegetable production largely depends on the availability of NO3, which is the most important N source (Colla et al. 2011). Excessive N use can reduce leaf quality because it causes nitrate accumulation in the leaves of vegetables with a short vegetation period such as lettuce (Awaad et al. 2016). Pseudomonas, Bacillus, Lactobacillus, Paenibacillus and Pantoea bacteria are root bacteria that stimulate plant growth (Chen et al. 1996; Fálico et al. 2000; Luz 2000; Pal et al. 2000; Wall 2000). Research has focused particularly on the genera Pseudomonas and Bacillus (Compant et al. 2005). It has been reported that many Pseudomonas species promote rapid growth by affecting seed germination, growth and root development, and Bacillus megaterium helps in the solubilization of P and K in soil (Wu et al. 2012; Keshavarz Zarjani et al. 2013; Sindhu et al. 2016; Zhao et al. 2019) and promotes plant growth (Zou et al. 2010; Zhou et al. 2016; Korir et al. 2017). In one study, it was found that biofertilizers enriched with inorganic fertilizers played an important role in the growth and yield of tomatoes and reduced inorganic fertilizer costs by 50% (Haque et al. 2012). As an alternative approach, NPK fertilizers were reduced by 75% by the use of microbial fertilizers in bean cultivation (Chauhan and Bagyaraj 2015). Zhao et al. (2021) *B. megaterium*, in combination with conventional fertilization increased cucumber yield by 11.8%-15.2%. Cucumber yield and quality were not negatively affected when P and K were reduced from conventional fertilization. In another study, microorganisms significantly increased the weight of red lettuce and contributed to the highest vitamin C concentration (Stojanović et al. 2020).

This research was conducted to determine the effects of a microbial fertilizer called Herasim, which contains a cocktail of different beneficial bacteria, on the growth, quality features and yield of curly lettuce grown in pots.

MATERIALS AND METHODS

Materials

Research Area and Growing Medium

This research was carried out by growing curly lettuce in pots in a glass greenhouse located at Akdeniz University, Turkiye. Before planting the seedlings, the pots were filled with a mixture of 30% perlite and 70% peat. The salt content of the peat used is very low, the grain size is between 0-7 mm and the pH value is 6.

Plant Material

Caipira curly lettuce variety was used as plant material in the research and seedlings were purchased from a seedling company. Caipira curly lettuce variety is suitable for open fields, greenhouses, vertical farming and hydroponic systems. The head structure of Caipira, which is a curly, dark green lettuce variety, is homogeneous; the leaves are thick, juicy and crisp. The variety, which is suitable for spring and early autumn production in the open field in temperate coastal regions, is also suitable for late autumn, winter and early spring cultivation under cover. Especially in cold periods, due to the high number of leaves, it looks as if it has reached the harvest stage, which facilitates its sale. The maturity period of the variety varies between 50-60 days in hot periods and 70-90 days in cool periods. The average head weight at the harvest stage is between 750-1100 g. Caipira lettuce variety is resistant to the 16th-26th, 28th and 32nd strains of lettuce mildew, to lettuce mosaic virus and aphids.

Microbial Fertiliser Used in The Research

The microbial fertiliser used in the experiment is a product called ‘Herasim Microbial Fertiliser’ belonging to Herasim Agricultural Products Livestock and Environmental Sciences Ltd. Co. Herasim Microbial Fertiliser contains *Lactobacillus lactis*, *Lactobacillus rhamnosus*, *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Rhodopseudomonas* *palustris*, *Bacillus subtilis bacteria and Kluyveromyces lactis* and *Saccharomyces cerevisiae* yeasts. It also contains organic carob molasses and water filtered by activated carbon and sand filters. Herasim microbial fertiliser contains 1x10^7 cfu/ml total number of live microorganisms. The content of Herasim microbial fertiliser is shown in Table 1.

Table 1. Content of Herasim microbial fertiliser

Content
Lactobacillus lactis
Lactobacillus rhamnosus
Lactobacillus plantarum
Lactobacillus acidophilus
Lactobacillus casei
Rhodopseudomonas palustris
Bacillus subtilis
Kluyveromyces lactis
Saccharomyces cerevisiae
Organic carob molasses
Activated carbon
Sand-filtered water

Climatic Characteristics of The Research Area

A data logger was placed in the research greenhouse to record humidity and temperature values. After the seedlings were planted, the average humidity was recorded as 58.72% in November, 68.84% in December, and 70.42% in January. The average temperature was measured as 19.52 °C in November, 17.10 °C in December, and 15.26 °C in January. The humidity and temperature values obtained are shown in Figure 1.

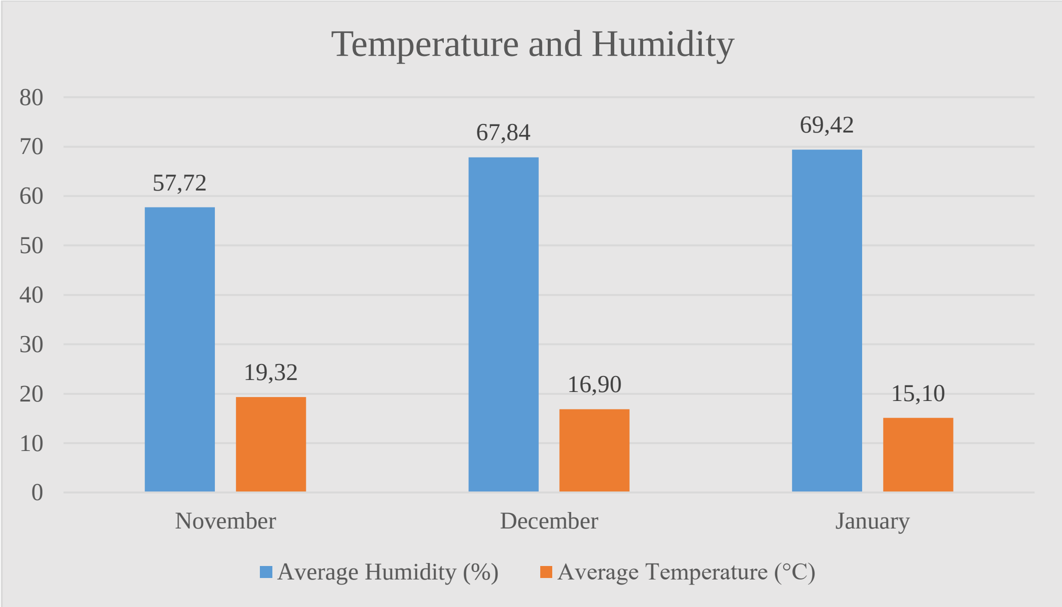


Figure 1. Humidity and temperature values recorded in the research greenhouse.

Properties of Irrigation Water Used in The Research

Well water has been used as irrigation water in the Faculty of Agriculture Research Area. Table 2 gives the pH and hardness of the irrigation water used in the research.

Table 2. Analysis of irrigation water

Parameters*	Value	Unit
pH	7.50	
EC (25 °C)	6.36	µmhos/cm
Bicarbonate (HCO_3)	5.44	meq/l
Calcium (Ca)	4.63	meq/l
Magnesium (Mg)	1.07	meq/l

*Irrigation water characteristics were used together with climate characteristics in another study conducted in the same greenhouse.

Method

Experiment Design and Treatments

Within the scope of the research, 9 different treatments were included together with the control treatment. The study was carried out according to the random plots experimental design with 3 replications. Lettuce seedlings were transplanted as one seedling for each pot on 04.11.2022. Chemical and microbial fertiliser applications were applied for lettuce plants in pots by calculating per plant. Chemical fertiliser treatments were calculated per plant in accordance with the recommended doses of 6.5 kg/da N, 2.5 kg/da P_2O_5, 6 kg/da K_2O as suggested by Aybak (2002). Chemical fertiliser was applied once a week on a certain day. Soil microbial fertiliser treatments were applied every 15 days on a certain day. Foliar microbial fertiliser treatments were applied once a week on a certain day. The treatments and their abbreviations are given in Table 3.

Table 3. Treatments and abbreviations included in the research

Treatments	Abbreviations
Control	C
100% Chemical Fertilisation	100% CF
50% Chemical Fertilisation+ Microbial Fertilisation	50% CF + MF
75% Chemical Fertilisation+ Microbial Fertilisation	75% CF + MF
100% Chemical Fertilisation+ Microbial Fertilisation	100% CF + MF
Immersion + Chemical Fertilisation	I + CF
Only Microbial Fertilisation	MF
50% Chemical Fertilisation+ Microbial Fertilisation + Foliar Microbial Fertilization	50% CF + MF + FMF
75% Chemical Fertilisation+ Microbial Fertilisation + Foliar Microbial Fertilization	75% CF + MF + FMF

Application of Microbial Fertilizer

Before lettuce seedlings were planted, a solution was prepared by mixing 1 litre of microbial fertiliser (1 L/60 L) in 60 litres of water. Except for the lettuce seedlings to be planted in the control and chemical fertiliser alone treatments, lettuce seedlings to be planted in the pots of the other treatments were planted after being immersed in the prepared solution for 30 min. After the seedlings were planted, the first irrigation was performed. One week after planting, microbial fertiliser and chemical fertiliser applications were started according to the research plan.

During the growing period, Herasim was applied with the irrigation water once every 15 days as 1 litre Herasim (1 L/60 L) per 60 litres of water. Herasim was also applied weekly by foliar spraying as 1 liter of Herasim in 120 liters of water (1 L/120 L).

Criteria Examined in Curly Lettuce

Curly lettuce plants reaching harvest maturity were harvested on 25 January 2023. The parameters measured and analysed in lettuce plants are given below. Total chlorophyll content was determined by SPAD 502 chlorophyll meter from the leaves of curly lettuce plants before harvesting. Harvested lettuce plants were crushed with a juicer and their juices were separated, and the TSS value were determined as % with a Hanna HI 96801 Model digital refractometer. Harvested lettuce plants were crushed with a juicer and their juices were extracted, and the pH of these juices was measured using a Hanna HI 2002-02 Model pH meter.

Colour measurements were taken as L, a* and b* on the third leaves from the outside of the harvested plants using the Minolta CR 400 Model colour chromameter, and the C (Chroma) and hº (hue) angle values were calculated using the *a and *b values.

C: $\sqrt{(a^2+b^2)}$

h (˚): $\tan^{-1}$ (b/a)

The L*, a* and b* colour parameters show the colour values that the human eye can perceive, and L* shows the changes in the brightness of the colour, reaching a maximum value as it approaches 100, which is called white. Of the colour values, a* shows the changes from green to red, and b* shows the changes from yellow to blue. Increasingly negative or positive values of the measured values indicate darkening of the colour, positive values of a* indicate red, negative values indicate green, and in the same way positive values of b* indicate yellow and negative values indicate blue. The hue angle calculated using the determined colour values indicates 0=red, 90=yellow, 180=green and 270=blue (Siomas et al. 2002; Madeira et al. 2003).

Statistical Analysis

The study was conducted according to the random plots trial design with 3 replications. The statistical analysis of the results was carried out with SAS 2009 Package Program and JPM Pro 17, an SAS program.

Figure 2. Lettuce plants in pot trials before harvest

RESULTS AND DISCUSSION

The effects of microbial fertiliser applications on lettuce head length (cm), root collar diameter (mm), number of leaves (number/plant), total soluble solids (TSS; %) and pH in lettuce juices are given in Table 4.

Table 4. Effects of microbial fertiliser applications on head length, root collar diameter, leaf number, TSS and pH of lettuce

Treatments	Head Length (cm)	Root Collar Diameter (mm)	Leaf Number (leaves/plant)	TSS (%)	pH
C	12.47 c*	14.92	39.00 b*	5.07 a*	6.23
100% CF	**18.33 a**	15.02	42.00 ab	4.20 b	6.21
50% CF + MF	17.03 ab	15.71	41.00 ab	4.03 b	6.22
75% CF + MF	**19.30 a**	15.43	42.00 ab	3.95 b	6.19
100% CF + MF	18.27 a	15.76	44.00 ab	3.87 b	6.17
I + CF	18.17 a	16.38	46.33 a	3.90 b	6.15
MF	14.33 bc	15.57	39.67 b	4.42 b	6.27
50% CF + MF + FMF	**19.53 a**	15.51	46.00 a	3.90 b	6.31
75% CF + MF + FMF	18.13 a	16.83	43.33 ab	3.97 b	6.25
$LSD_{\%5}$	2.7746	N.S.	5.8963	0.5836	N.S.

*Differences between values not indicated with the same letter are significant at p<0.05 level.
N.S: Not Significant

Different microbial fertilizer applications had significant effects on head length, leaf number and TSS, but no significant effects on root collar diameter and pH ($p<0.05$). The shortest plants in terms of head length were measured in the C and MF plots. The Plants in other treatments were evaluated in the same group. However, the highest values were obtained from 50% CF + MF + FMF, 75% CF + MF, 100% CF, I + CF and 75% CF + MF + FMF treatments. Root collar diameter values varied between 16.83-14.92 cm and there were no significant differences between treatments. When the number of leaves was compared in different treatments, the plants with the lowest number of leaves were obtained in the C and MF treatments, while there were no significant differences in between the other treatments, but more leaves were obtained compared to the C and MF treatments. Total soluble solids content was highest in C and all other treatments were in the same group with lower values. There were no significant differences between the measured pH values and they varied between 6.31-6.15.

Microbial fertilizer applications combined with inorganic fertilizer did not negatively affect the growth characteristics of lettuce and even showed an improving effect. These results are consistent with previous studies. For example, Demir et al. (2023) reported similar results in terms of head length, leaf number and TSS of lettuce with the use of a microbial fertilizer in combination with chemical fertilisers. In addition, contrary to this study, it was reported that it was effective on root collar diameter and pH. Miskoska-Milevska et al. (2018) found that commercial Slavol containing the microorganisms *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Derxia sp.*, *B. megaterium*, *B. licheniformis* ve *B. subtilis* increased leaf length and width when sprayed on cauliflower leaves. On the contrary, Rabiei et al. (2020) reported that *Azospirillum brasilense* and *Azotobacter chroococcum* did not affect the length of coriander whose leaves were consumed. Polat et al. (2004) reported that TSS was in the range of 3.86%-4.06% in head lettuce and 4.40%-4.60% in cos type lettuce and pH was in the range of 5.99-6.06% in head lettuce and 5.89-5.94% in cos type lettuce. These values are compatible with our study. Özbay et al. (2010) found that *T. harzianum* bacteria increased the TSS of rocket by 13% and cress by 11%.

The effects of microbial fertilizer applications on L*, C and hº colour values and total chlorophyll (SPAD) content of lettuce are shown in Table 5.

Table 5. Effects of microbial fertilizer applications on L*, C and hº colour values and total chlorophyll of lettuce

Treatments	L*	C	hº	Chlorophyll
C	66.74 a*	43.26 a*	116.25 c*	24.60 ed*
100% CF	59.68 bc	40.93 cd	118.95 ab	31.47 a
50% CF + MF	58.39 cd	42.06 abc	119.24 a	26.53 cde
75% CF + MF	56.68 cd	40.61 cd	119.70 a	30.07 ab
100% CF + MF	57.53 cd	41.66 abcd	119.24 a	27.83 bcd
I + CF	59.96 bc	41.28 bcd	119.25 a	30.80 ab
MF	63.68 ab	42.96 ab	117.84 b	23.90 e
50% CF + MF + FMF	58.08 cd	40.95 cd	118.79 ab	29.50 abc
75% CF + MF + FMF	55.15 d	40.16 d	119.41 a	31.07 ab
$LSD_{\%5}$	4.4066	1.7997	1.2615	3.3217

*Differences between values not indicated with the same letter are significant at p<0.05 level.
N.S: Not Significant

Statistically significant differences were found between treatments in terms of colour criteria and chlorophyll content (p<0.05). The highest L* value in lettuce leaves was measured in C and MG. The lowest L* value was determined in 75% CF + MF + FMF, 75% CF + MF, 100% CF + MF, 50% CF + MF + FMF and 50% CF + MF plots. The highest C value indicating the saturation in leaf colour was calculated in C and MG, while the lowest values were calculated in 75% CF + MF + FMF, 75% CF + MF, 100% CF and 50% CF + MF + FMF treatments. The hº angle, which expresses the changes in the colour of the leaves, was highest in 75% CF + MF, 75% CF + MF + FMF, I + CF, 50% CF + MF, 100% CF + MF and 50% CF + MF + FMF treatments. The lowest hº value was determined in K. Green colour is an important morphological trait in lettuce, the leaves of which are consumed as a vegetable. When the colour values obtained were examined from this point of view, it can be said that greener lettuce was harvested in 50% CF + MF, 75% CF + MF, 100% CF + MF, I + CF, 50% CF + MF + FMF and 75% CF + MF + FMF treatments. When the total chlorophyll content was evaluated according to the treatments, the highest values were obtained in the plants in 100% CF, 75% CF + MF + FMF, I + CF and 75% CF + MF plots. The treatments with the lowest chlorophyll content were in the MF and C plots. It was also determined that the chlorophyll contents were generally in parallel with the colour values.

Figure 3. 75% CF + MF + FMF treatment plants that have reached the harvest stage.

Ucok et al. (2019) showed in their research with different organic fertilisers that L* in curly lettuce varied between 56.23–59.43, C varied between 37.19–38.82, and hº varied between 115.56–117.61. Contrary to these, Sönmez et al. (2017) found that organic fertilisers did not affect these colour values. The colour values determined in our research are consistent with these studies. Demir et al. (2023), who investigated the effects of microbial fertilisers on lettuce, reported quite similar results to our research in terms of the lettuce colour and chlorophyll changes.

The effects of different microbial fertiliser applications on total (g/plant) and marketable (g/plant) yield of lettuce are shown in Table 5.

Table 5. Effects of different microbial fertilizer applications on total and marketable yield of curly lettuce.

Treatments	Total yield (g/plant)	Marketable yield (g/plant)
C	138.33 c*	125.00 c*
100% CF	270.00 ab	246.67 ab
50% CF + MF	253.33 b	235.00 b
75% CF + MF	280.00 ab	260.00 a
100% CF + MF	281.67 ab	266.67 ab
I + CF	296.67 a	276.67 a
MF	146.67 c	140.00 c
50% CF + MF + FMF	278.33 ab	261.67 ab
75% CF + MF + FMF	286.67 a	261.67 ab
$LSD_{\%5}$	31.622	32.43

*Differences between values not indicated with the same letter are significant at p<0.05 level.

Different treatments significantly affected total and marketable lettuce yield (p<0.05). The highest total yields were found in I + CF (296.67 g/plant), 75% CF + MF + FMF (286.67 g/plant), 100% CF + MF (281.67 g/plant), 75% CF + MF (280.00 g/plant), 50% CF + MF + FMF (278.33 g/plant) and 100% CF (270.00 g/plant) treatments. The lowest yield values were determined in C (138.33 g/plant) and MF (146.67 g/plant) treatments. The highest values in terms of marketable yield were obtained from I + CF (276.67 g/plant), 100% CF + MF (266.67 g/plant), 75% CF + MF + FMF (261.67 g/plant), 50% CF + MF + FMF (261.67 g/plant), 75% CF + MF (260.00 g/plant) and 100% CF (246.67 g/plant) treatments. Similar to the total yield, the lowest marketable yields were again determined in C (125.00 g/plant) and MF (140.00 g/plant) treatments.

When the results were evaluated in terms of yield; in the I + CF treatment, which gave the highest value in total yield, 114.5% more yield was obtained compared to C and 102.3% more yield was obtained compared to MF. When this application (I + CF) was compared to 100% CF, 9.9% more total yield was obtained. The 75% CF + MF + FMF treatment, provided the highest yield although the chemical fertilizer dose was reduced by 25%; this yield is 107.2%

higher than that of C; 95.5% higher than that of MF and 6.2% more than the yield obtained in 100% CF.

There are many studies supporting the view that microbial fertilisers increase the yield and reduce chemical fertiliser consumption. Demir et al. (2023) found the highest total yield in lettuce in the microbial fertiliser, “immersion + chemical fertilisation,” and in the “chemical fertilisation (CF) + microbial fertiliser (MF)” applications, while in the same study, the highest first class yield in cucumber was obtained in 50% CF + MF application. When the researchers compared the results obtained, they reported that savings could be achieved on chemical fertilisers by introducing accompanying microbial fertilisation. These results are similar to our research. Vejan et al. (2016) reported that yield was increased between 7% and 33% with the use of PGPR. Stojanovic et al. (2020) concluded that some microorganisms were effective on lettuce yield. There are other studies with similar results on saving on chemical fertiliser. For example, Chauhan and Bagyaraj (2015) found that NPK in beans could be reduced by microbial inoculation. Kafi et al. (2021) determined that the recommended NPK dose in cucumber could be reduced by 30% by utilizing some beneficial bacteria.

The positive and negative proportional effects related to the criteria examined within the scope of the research are shown with Pearson correlation coefficients (Table 6). Average values were used in the criteria examined.

Table 6. Pearson correlation coefficients related to the criteria examined in the study.

Parameters	L	C	hº	Chlorophyll	Total Yield	Market. Yield	Head Length	Root Collar Diam.	Leaves Num.	TSS	pH
L	1,000										
C	0,888**	1,000									
hº	-0,935**	-0,817**	1,000								
Chlorophyll	-0,732*	-0,935**	0,722*	1,000							
Total Yield	-0,898**	-0,884**	0,902**	0,877**	1,000						
Market. Yield	-0,897**	-0,867**	0,907**	0,855**	0,998**	1,000					
Head Length	-0,897**	-0,893**	0,904**	0,835**	0,946**	0,953**	1,000				
Root Collar Diam.	-0,585^{ns}	-0,486^{ns}	0,537^{ns}	0,392^{ns}	0,502^{ns}	0,501^{ns}	0,356^{ns}	1,000			
Leaves Num.	-0,609^{ns}	-0,652^{ns}	0,607^{ns}	0,699*	0,807**	0,823**	0,772*	0,525^{ns}	1,000		
TSS	0,904**	0,761*	-0,952**	-0,661^{ns}	-0,897**	-0,916**	-0,919**	-0,571^{ns}	-0,766*	1,000	
pH	0,134^{ns}	0,095^{ns}	-0,320	-0,268^{ns}	-0,351^{ns}	-0,349	-0,160^{ns}	-0,136^{ns}	-0,157^{ns}	0,20^{ns}	1,000

*Significant at p<0.05 level; **Significant at p<0.001 level; ns: Not Significant

There was a strong positive relationship between L and C and TSS among the examined criteria, while there was a strong negative relationship between L and hº, total yield, marketable yield and head length. The negative relationship between L and chlorophyll was moderate. The significant relationships between C and other criteria were negative except for TSS (positive). Among the relationships between hº and other important criteria, only TSS was negative. There was a strong positive relationship between chlorophyll and total yield, marketable yield, head length, and a moderately significant positive relationship with the number of leaves. While there was a strong positive relationship between total yield and marketable yield, head length and number of leaves, there was a strong negative relationship with TSS. The relationships between the examined criteria can be seen in Table 6.

The principal component analysis conducted by considering the criteria examined in the research is shown in Figure 4, and the load matrices are shown in Table 7.

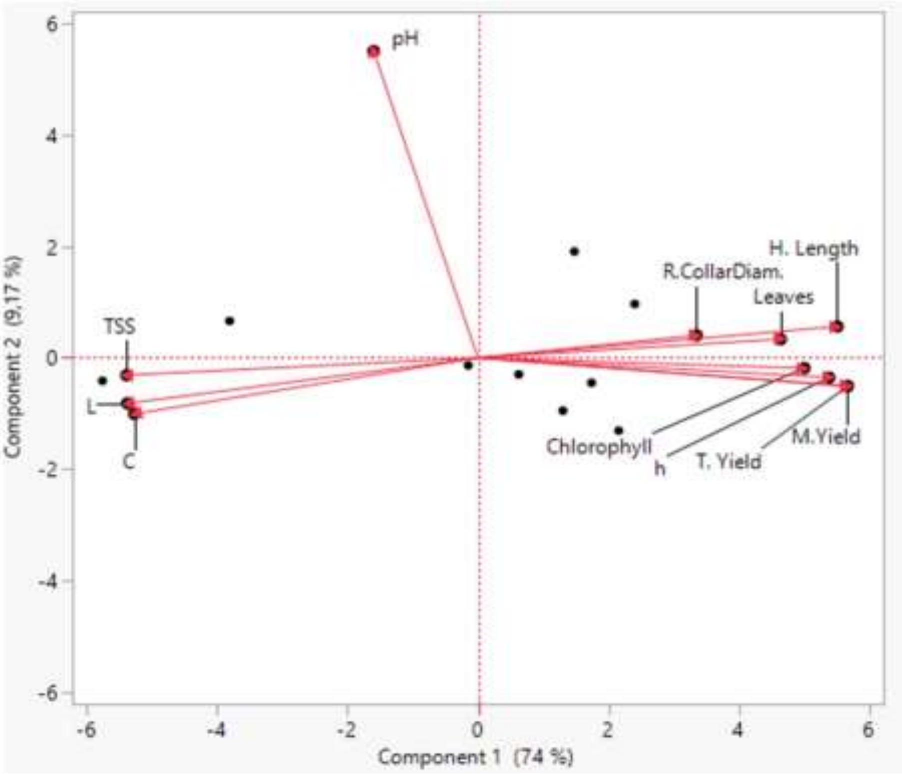


Figure 4. Principal component analysis results of the parameters examining the effects of microbial fertilizer applications.

According to the principal component analysis results, Figure 4 shows two components. The first principal component represented 74%, the second principal component 9.17% and the total variation 83.17%. All analysed components and factor loadings can be seen in Table 7.

Table 7. Load matrices of the examined criteria

Parameters	Prin1	Prin2	Prin3	Prin4	Prin5
L	-0,93390	-0,14187	-0,05527	0,28158	0,05680
C	-0,91533	-0,17470	0,15446	-0,01020	0,31765
H	0,93532	-0,06099	0,03716	-0,31904	0,05801
Chlorophyll	0,86920	-0,03195	-0,25100	0,23750	-0,33756
Total Yield	0,98398	-0,08892	-0,08128	0,04446	0,02241
Marketable Yield	0,98470	-0,08739	-0,06865	0,04093	0,07833
Head Length	0,95707	0,09793	-0,23209	-0,04192	0,12400
Root Collar Diameter	0,58232	0,07031	0,78301	0,07432	-0,19250
Leaves Number	0,80591	0,05831	0,08860	0,50667	0,28424
TSS	-0,93574	-0,05393	-0,11003	0,15663	-0,27603
pH	-0,27789	0,95777	-0,04517	0,01212	0,03662

The most important traits constituting the first main component were marketable yield, total yield, head length, TSS, h colour value, L* and C colour values, chlorophyll and leaf number according to factor loadings. In the second main component, only the most important factor loading was pH, while the third component was root collar diameter.

CONCLUSIONS

This research was carried out in pots to determine the effects of a microbial fertiliser called Herasim containing different beneficial bacteria in a cocktail form on the growth, quality and yield characteristics of lettuce. The results were obtained in the study were significant. First of all, it was determined that Herasim microbial fertiliser had no negative effects on lettuce growth characteristics, quality criteria, leaf colours and yield. It was determined that basically similar results were obtained with 100% CF in terms of the criteria examined, and even some healing effects of microbial fertilizer appeared when the chemical fertilizer dose was reduced. For example, greener lettuce leaves were harvested by applying Herasim at reduced chemical fertiliser doses.

In addition to different chemical fertiliser doses, higher yield values were obtained with microbial fertilisers applied in comparison to that of control and microbial fertiliser only applications. Despite the reduced NPK doses, it is noteworthy that similar yield results were obtained with 100% CF alone with the addition of microbial fertiliser. In this respect, 75% CF + MF and 75% CF + MF + FMF and 50% CF + MF + FMF trials are important in terms of fertiliser savings. The increase in yield by spraying microbial fertiliser on the leaves was also found to be remarkable.

We believe that it is no coincidence that the highest total yield (296.67 g/plant) and marketable yield (276.67 g/plant) were obtained in the I + %100 CF application where Herasim Microbial Fertiliser was applied only by the immersion method on lettuce before transplanting because in this treatment the microbial application was carried out at the earliest stage and directly to the roots of the plant. As a direct result of this, it should be understood that the plant could probably develop better as a result of better uptake of nutrients from the plant roots at an early stage in this treatment.

In view of the above study, we highly recommend the use of Herasim Microbial Fertiliser to save on Chemical Fertilisers.

ACKNOWLEDGEMENTS

We would like to thank Herasim Agriculture and Livestock Products Ltd. Co. for providing the Herasim Microbial Fertilizer support used in the research.

REFERENCES

Aćamović-Đoković, G., Pavlović, R., Mladenović, J., and Djurić, M. 2011. Vitamin C content of different types of lettuce varieties. Acta Agriculturae Serbica, 16(32), 83–89.

Aksoy, U., Altındişli, A. 1998. Ekolojik (Organik, Biyolojik) Tarım. ETOD, 125s, İzmir.

Aksoy, U. 1999. Ekolojik Tarımdaki Gelişmeler. Ekolojik Tarım, Ekolojik Tarım Organizasyonu Derneği, Emre Basımevi, 30-35s. İzmir.

Antoun, H., ve Prévost, D. 2005. Ecology of plant growth promoting rhizobacteria. In Z. A. Siddiqui (Ed.), PGPR: Biocontrol and biofertilization (pp. 1–38). Dordrecht, Netherlands: Springer.

Awaad, M. S., Badr, R. A., Badr, M. A., and Abd-Elrahman, A. H. 2016. Effects of different nitrogen and potassium sources on lettuce (Lactuca sativa L.) yield in a sandy soil. Eurasian Journal of Soil Science, 5(4), 299–306, doi: 10.18393/ ejss.2016.4.299-306.

Aybak H.Ç. (2002). *Salata/Marul Yetiştiriciliği*. ISBN: 975-8377-20-5, (sayfa sayısı 96), İstanbul: Hasad Yayıncılık (in Turkish).

Chauhan, H., and Bagyaraj, D. J. 2015. Inoculation with selected microbial consortia not only enhances growth and yield of French bean but also reduces fertilizer application under field condition. Scientia Horticulturae, 197, 441–446, doi: 10.1016/j. scienta.2015.10.001.

Chen, Y., Mei, R., Lu, S., Liu, L., and Kloepper, J. W. 1996. The use of yield increasing bacteria (YIB) as plant growth promoting rhizobacteria in Chinese agriculture. In R. S. Uthkede, and W. K. Gupta (Eds.), Management of soil borne diseases (pp. 164–184). Ludhiana: Kalyani Publishers.

Chen. J. H. 2006. The combined use of chemical and organic fertilizers and/or biofertiliser for crop growth and soil fertility, International Workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use, 16 – 20 October, Land Development Department, Bangkok, Thailand.

Colla, G., Suárez, C. M. C., Cardarelli, M., and Rouphael, Y. 2010. Improving nitrogen use efficiency in melon by grafting. HortScience, 45(4), 559–565, doi: 10.21273/HORTSCI.45.4.559.

Colla, G., Rouphael, Y., Mirabelli, C., and Cardarelli, M. 2011. Nitrogen-use efficiency traits of miniwatermelon in response to grafting and nitrogen fertilization doses. Journal of Plant Nutrition and Soil Science, 174, 933–941, doi: 10.1002/jpln. 201000325.

Colonna, E., Rouphael, Y., Barbieri, G., and De Pascale, S. 2016. Nutritional quality of ten leafy vegetables harvested at two light intensities. Food Chemistry, 199, 702–710, doi: 10.1016/j. foodchem.2015.12.068.

Compant, S., Duffy, B., Nowak, J., Clement, C., and Barka, E. A. 2005. Use of plant growth promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action, and future prospects. Applied and Environmental Microbiology, 71(9), 4951–4959, doi: 10.1128/ AEM.71.9.4951-4959.2005.

Demir H., Yalci H.K., Katgici A. 2023. Ameliorative effects of microbial fertiliser on yield and quality parameters of curly lettuce and cucumber with fertiliser saving. Folia Horticulturae, 35 (1): 91-106. https://doi.org/10.2478/fhort-2023-0007

Fálico, L., García, B., Sillón, M., and Visintín, G. 2000. Bacillus spp. and growth promotion in soybean. Proceedings, Fifth International PGPR Workshop, 29 October-3 November, p. 40, CordobaArgentina

Goessler, W., & Kuehnelt, D. (2002). Analytical methods for the determination of arsenic and arsenic compounds in the environment. Environmental chemistry of arsenic, 27-50.

Gravel, V., Anton, H., and Tweddell, R. J. 2007. Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with Pseudomonas putida or Trichoderma atroviride: Possible role of indole acetic acid (IAA). Soil Biology and Biochemistry, 39, 1968–1977, doi: 10.1016/j. soilbio.2007.02.015.

Gupta, A. ve Sen, S. 2013. Role of biofertilisers and biopesticides for sustainable agriculture, scholar.google.com.

Haque, Md. M., Ilias, G. N. M., and Molla, A. H. 2012. Impact of Trichoderma-enriched biofertilizer on the growth and yield of mustard (Brassica rapa L.) and tomato (Solanum lycopersicon Mill.). The Agriculturists, 10(2), 109–119.

Kafi, S. A., Arabhosseini, S., Karimi, E., Koobaz, P., Mohammadi, A., and Sadeghi, A. (2021). Pseudomonas putida P3-57 induces cucumber (Cucumis sativus L.) defense responses and improves fruit quality characteristics under commercial greenhouse conditions. Scientia Horticulturae, 280, 109942, doi: 10.1016/j.scienta.2021.109942.

Kengo, Y., and Hui-Lian, X. 2000. Properties and applications of an organic fertilizer inoculated with effective microorganisms. Journal of Crop Production, 3(1), 255–268, doi: 10.1300/ J144v03n01_21.

Keshavarz Zarjani, J., Aliasgharzad, N., Oustan, S., Emadi, M., and Ahmadi, A. 2013. Isolation and characterization of potassium solubilizing bacteria in some Iranian soils. Archives of Agronomy and Soil Science, 59(12), 1713–1723, doi: 10.1080/03650340.2012.756977.

Korir, H., Mungai, N. W., Thuita, M., Hamba, Y., and Masso, C. 2017. Co-inoculation effect of Rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Frontiers in Plant Science, 8, 141, doi: 10.3389/ fpls.2017.00141

Lévai, L., Veres, S. Z., Makleit, P., Marozsán, M., and Szabó, B. 2006. New trends in plant nutrition. Proceedings of the 41st Croatian and 1st International Symposium on Agriculture, Opatija, Croatia, 13–17 February, 435–436, ISBN 953-6331-39-X.

Luz, W. C. 2000. Plant Growth Promoting Rhizobacteria in Graminicolus crops in Brazil. Paper presented at Fifth International PGPR Workshop, 29 October–3 November, Cordoba, Argentina.

Madeira, A.C., Ferreira, A., de Varennes, A., and Vieira, M.I. (2003). SPAD Meter Versus Tristimulus Colorimeter to Estimate Chlorophyll Content and Leaf Colour in Sweet Pepper. *Communications in Soil Science and Plant Analysis*, *34*(17-18), 2461-2470, doi: 10.1081/CSS-120024779.

Mĭskoska-Mĭlevska, E., Najdenovska, O., Popovskï, Z. T., and DimovsD. (2018). The influence of the microbiological fertilizer–Slavolon cauliflower growth. Romanian Biotechnological Letters, 23(2), 13511–13516.

Molla, A. H., Haque, M. M., Haque, M. A., and Ilias, G. N. M. 2012. Trichoderma-enriched biofertilizer enhances production and nutritional quality of tomato (Lycopersicon esculentum Mill.) and minimizes NPK fertilizer use. Agricultural Research, 1(3), 265–272, doi: 10.1007/s40003-012-0025-7.

Muraleedharan, H., Seshadri, S ve Perumal, K. 2010. Biofertiliser (Phosphobacteria), Shri Murrugapa Chettiar Research Centre.

Ozbay, N., Emrebas, N., and Akinci, S. 2010. Effects of a microbial fertilizer (Trichoderma hertzian, Kuen 1585) on growth and yield of arugula (Eruca vesicaria subsp. sativa) and garden cress (Lepidium sativum) grown in soilless culture. The Journal of Agricultural Faculty of Ege University, Special Issue, 268–274, ISSN 1018-8851.

Pal, K. K., Dey, R., Bhatt, D. M., and Chauhan, S. M. 2000. Plant Growth Promoting Fluorescent Pseudomonas Enhanced Peanut Growth, Yield And Nutrient Uptake. Paper presented at Fifth International PGPR Workshop, 29 October–3 November, Cordoba, Argentina.

Pei-Feng, S., Wei-Ta, F., Li-Ying, S., Jyuan-Yu, W., Shih-Feng, F., and Jui-Yu, C. 2014. Indole-3-acetic acid-producing yeasts in the phyllosphere of the carnivorous plant Drosera indica L. PLOS One, 9(12), e114196, doi: 10.1371/journal.pone.0114196

Parr, J.F., Hornick, S.B. And Kaufman, D.D. 1994. Use of Microbial Inoculants and Organic Fertilizers In Agricultural Production. http://www.agnet.org/Library/eb/394/eb394.pdf

Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., and Bakker, P. A. H. M. 2014. Induced systemic resistance by beneficial microbes. Annual Review of Phytopathology, 52, 347–375, doi: 10.1146/annurevphyto-082712-102340

Polat, E., Onus, A. N., and Demir, H. (2004). Atik Mantar Kompostunun Marul Yetiştiriciliğinde Verim ve Kaliteye Etkisi. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 17(2), 149–154.

Polat, E., Uzun, H. İ., Topçuoğlu, B., Önal, K., Onus, A. N., and Karaca, M. (2009). Effects of spent mushroom compost on quality and productivity of cucumber (Cucumis sativus L.) grown in greenhouses. African Journal of Biotechnology, 8(2), 176–180.

Prasanna, R., Sood, A., Ratha, S. K., and Singh, P. K. 2014. Cyanobacteria as a “green option” for sustainable agriculture. In N. K. Sharma, L. J. Stal, and A. K. Rai (Eds.), Cyanobacteria: An economic perspective (pp. 145–166). Chichester: Wiley

Rabiei, Z., Hosseini, S. J., Pirdashti, H., and Hazrati, S. (2020). Physiological and biochemical traits in coriander affected by plant growth-promoting rhizobacteria under salt stress. Heliyon, 6(10), e05321, doi: 10.1016/j.heliyon.2020.e05321.

Richardson, A. E., Barea, J., Mcneill, A. M., and Prigent-Combare, C. 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil, 321, 305–339, doi: 10.1007/s11104- 009-9895-2.

Sindhu, S. S., Parmar, P., Phour, M., and Sehrawat, A. 2016. Potassium-solubilizing microorganisms (KSMs) and its effect on plant growth improvement. In V. S. Meena, B. R. Maurya, J. P. Verma, and R. S. Meena (Eds.), Potassium solubilizing microorganisms for sustainable agriculture (pp. 171–185). India: Springer, doi: 10.1007/978-81-322- 2776-2_13.

Singh, D. S., Chand, S., Anvar, M., and Patra, D. D. 2003. Effect of organic and inorganic amendment on growth and nutrient accumulation by Isabgol (Plantago ovata) in sodic soil under greenhouse conditions. Journal of Medicinal and Aromatic Plant Sciences, 25(2), 414–419.

Siomas, A.S., Papadopoulou, P.P., and Gogras, C.C. (2002). Quality of Romaine and Leaf Lettuce at Harvest and during Storage. Proc.2nd Balkan Symposium on Vegetables and Potatoes. *Acta Horticulturae*, *579*, 641-646.

Sönmez, İ., Kalkan, H., Demir, H., Külcü, R., Yaldiz, O., and Kaplan, M. (2017). Mineral composition and quality parameters of greenhouse-grown lettuce (Lactuca sativa L.) depending on fertilization with agricultural waste composts. Acta Scientiarum Polonorum, Hortorum Cultus, 16(3), 85–95, doi: 10.24326/asphc.2017.3.9.

Stojanović, M., Petrović, I., Žuža, M., Jovanović, Z., Moravčević, D., Cvijanović, G., and Savić, S. 2020. The productivity and quality of Lactuca sativa as influenced by microbiological fertilisers and seasonal conditions. Zemdirbyste-Agriculture, 107(4), 345–352, doi: 10.13080/z-a.2020.107.044.

Triveni, S., Prasanna, R., Kumar, A., Bidyarani, N., Singh, R., and Saxena, A. K. 2015. Evaluating the promise of Trichoderma and Anabaena based biofilms as multifunctional agents in Macrophomina phaseolina-infected cotton crop. Biocontrol Science and Technology, 25(6), 656–670, doi: 10.1080/09583157.2015.1006171.

TÜİK 2024. Bitkisel Üretim İstatistikleri, 2023. https://data.tuik.gov.tr/Bulten/Index?p=Bitkisel-Uretim-Istatistikleri-2023-49535

Ucok, Z., Demir, H., Sonmez, I., and Polat, E. (2019). Effects of different organic fertilizer applications on yield, quality and plant nutrient content of curly salad (Lactuca sativa L. var. crispa). Mediterranean Agricultural Sciences, 32(Special issue), 63–68, doi: 10.29136/ mediterranean.559120.

Vejan, P., Abdullah, R., Khadiran, T., Ismail, S., and Boyce, A. N. (2016). Role of plant growth promoting rhizobacteria in agricultural sustainability - a review. Molecules, 21(5), 573, doi: 10.3390/ molecules21050573.

Vessey, J. K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant and Soil, 255, 571–586.

Wall, L. G. 2000. Consequences of an Overview on Plant Growth Promoting Rhizobacteria Work in Argentina: The Field Should be Wider. Paper presented at Fifth International PGPR Workshop, 29 October-3 November, Cordoba, Argentina.

Wang G, Ren Y, Bai X, Su Y, Han J. Contributions of Beneficial Microorganisms in Soil Remediation and Quality Improvement of Medicinal Plants. Plants (Basel). 2022 Nov 23;11(23):3200. doi: 10.3390/plants11233200. PMID: 36501240; PMCID: PMC9740990.

Wu, F., Wan Judy Hon, C., Wu, S., and Wong, M. 2012. Effects of earthworms and plant growth promoting rhizobacteria (PGPR) on availability of nitrogen, phosphorus, and potassium in soil. Journal of Plant Nutrition and Soil Science, 175, 423–433, doi: 10.1002/jpln.201100022.

Zhao, Y., Zhang, M., Yang, W., Di, H. J., Ma, L., Liu, W., and Li, B. 2019. Effects of microbial inoculants on phosphorus and potassium availability, bacterial community composition, and chili pepper growth in a calcareous soil: A greenhouse study. Journal of Soils and Sediments, 19, 3597–3607, doi: 10.1007/ s11368-019-02319-1

Zhao, Y., Mao, X., Zhang, M., Yang, W., Hong, J. Di., Ma, L., Liu, W., and Li, B. 2021. The application of Bacillus megaterium alters soil microbial community composition, bioavailability of soil phosphorus and potassium, and cucumber growth in the plastic shed system of North China. Agriculture, Ecosystems and Environment, 307, 107236, doi: 10.1016/j. agee.2020.107236.

Zhou, C., Ma, Z., Zhu, L., Xiao, X., Xie, Y., Zhu, J., and Wang, J. 2016. Rhizobacterial strain Bacillus megaterium BOFC15 induces cellular polyamine changes that improve plant growth and drought resistance. International Journal of Molecular Sciences, 17(6), 976, doi: 10.3390/ijms17060976.

Zou, C., Li, Z., and Yu, D. 2010. Bacillus megaterium strain XTBG34 promotes plant growth by producing 2-pentylfuran. The Journal of Microbiology, 48(4), 460–466, doi: 10.1007/s12275-010-0068-z.

STUDY OF THE INFLUENCE OF HARVESTING, IRRIGATION, AND FERTIGATION ON THE PHYSICOCHEMICAL AND BIOCHEMICAL PARAMETERS DURING COLD STORAGE OF WHITE STRAWBERRY FRUITS (*FRAGARIA X ANANASSA "SNOW WHITE"*) GROWN UNDER GREENHOUSE CONDITIONS IN BULGARIA

Elena GRANCHAROVA[1], Petya IVANOVA[2], Blagoj ELENOV[1], Stoil ZHELYAZKOV[2]

[1] Institute of soil science, agrotechnologies and plant protection „Nikola Poushkarov", Agricultural Academy, Bulgaria
[2] Institute of Food Preservation and Quality, Agricultural Academy, Bulgaria
Corresponding author e-mail: eveha@abv.bg

ABSTRACT

The aim of this paper is to present the effects of the time of harvesting, irrigation and fertigation on the physicochemical and biochemical indicators of white strawberry fruits during the cold storage (T= 4ºC).
The fruits were grown in 2024 as part of a two-factor experiment conducted in an experimental field at the Institute of Soil Science, Agrotechnology, and Plant Protection "Nikola Poushkarov," Chelopechene, Sofia, in an unheated greenhouse with drip irrigation and fertigation. The following irrigation and fertilization rates were applied: I1 – 75% (ETc), I2 – 50% (ETc), F1: optimal fertilization $N_{8.09}P_{12.76}K_{15.62}$, F2 – suboptimal fertilization – 75% (F1). Five variants were studied: control: I0F0: 100% (ETc) without fertilization; I1F1; I1F2; I2F1; I2F2.
The storage under refrigerated conditions (T = 4°C) and the analysis of fruit quality using standardized methods and adapted techniques over a period of 6 days were conducted in the laboratories of the "Food Technology" department at the Institute of Food Preservation and Quality – Plovdiv. The study dynamically examined the physicochemical parameters (soluble and insoluble dry matter, %; active acidity; moisture, %; water activity; chromatic parameters L*, a*, b*) and biochemical parameters (total polyphenols, mg GAE/100 g, and antioxidant activity, µmol TE/100 g) of the fruits during storage.
It was found that the time of harvest, cultivation method, and storage period under refrigerated conditions influenced the values of physicochemical parameters for all studied variants of white strawberry fruits (Fragaria x Ananassa "Snow White"), with the most significant impact on antioxidant activity and total polyphenol content ($p > 0.05$).

Keywords: White strawberry, Physicochemical parameters, Color characteristics, Antioxidant activity, Total polyphenols, Irrigation, Fertigation, Greenhouse cultivation,

INTRODUCTION

Strawberries with white fruits have long history of cultivation no shorter then red varieties. White strawberry has cultivated for hundreds of years in Chile and has grown in two botanical forms - wild *Fragaria chiloensis ssp. chiloensis f. patagonica* and cultivated *Fragaria chiloensis ssp. chiloensis f. chiloensis* (Grez et al., 2020). It was brought to Europe in the 18th century. „Snow White" cultivar has been selected in 2010 out from *Fragaria x ananassa*

"Weisse Ananas" and *Fragaria chiloensis f. Chiloensis* (Olbricht et al., 2013). White strawberries are distinguished by their white or pale pink flesh and unique pineapple-like flavor.

Fragaria Ananassa berries are an important source of vitamins, minerals and fibers. The diversity of bioactive compounds, such as phenols in berry species, is reflected in a wide range of their biological activities, which have a beneficial effect on human health and disease prevention (Moyer, R, 2002). The activity of phenols is expressed in antioxidant (Cirico et al., 2006), neuroprotective (Galli et al., 2006) anticancer (Ramirez - Tortosa et al., 2001; Markovic et al., 2000), anti-inflammatory (Karakaya et al., 2004) and antimicrobial activity. The physicochemical and biochemical parameters of red variety strawberry have been widely studied (Sadik et al. 2023; Cordenunsi et al. 2003; Kumar et al. 2021; Muley et al. 2022; Wigati et al. 2024; Pinheiro et al. 2021; Balan et al., 2024), while white variety has been still limited (Ivanova et al., 2024).

This research investigates the effects of drip irrigation and fertigation on white strawberries' physical characteristics during cold storage.

MATERIAL AND METHOD

✓ Experimental layout

The second year two-factor experiment was conducted on drip irrigated strawberry plants in a tunnel greenhouse in 2023 and 2024 in the Chelopechene experimental field (latitude 42°44′22.8″N, longitude 23°28′3.7″E and altitude 550 m above sea level) of the Institute of soil science, agrotechnologies and plant protection "Nikola Poushkarov" in Sofia, Bulgaria. Sofia field falls into temperate continental climate subzone. The greenhouse was unheated with area 420 m^2 (7.9 m x 53 m) covered with a five-layer UV+EVA+IR+AD+dif -150 μm polyethylene film. The soil could be defined as moderate to strong water-permeable with an average filtration capacity. The soil was *Chromic Luvisol* with bulk density 1.47 g cm^{-3}, field capacity 22% and wilting point 10% for 0-50 cm layer. To further reduce water losses, mulching with silver-black UV polyethylene mulch with a thickness of 30 μm was applied.

The object of the study was white strawberry cultivar (*Fragaria × ananassa "Snow White"*). The experimental treatments were arranged according to the method of long plots with three replications. Each plot has 23.2 m^2 area and consisted of twin rows of strawberries. Healthy bare-root frigo plants were planted in scheme of 90 + 30/30 cm on 22 March 2023. According to the white strawberries cultivation technology in each of the experimental plots were provided the appropriate amount of red fruit plants (4: 1 ratio) to ensure better pollination. The irrigation factor was applied in two rates: I1 – deficit irrigation - 75% (ETc); I2 - deficit irrigation - 50% (ETc). The fertilization factor was applied in two rates: F1: optimal fertilization $N_{8.09}P_{12.76}K_{15.62}$; F2 – suboptimal fertilization - 75% (F1) - $N_{6.07}P_{9.57}K_{11.94}$. Optimal fertilization was developed according to Haifa nutrition recommendations (Haifa Group, 2021) as follows: Haifa MAP- 25-45 kg ha^{-1} , Multi K – 80 kg ha^{-1} , Haifa MKP - 25 kg ha^{-1} , Haifa Cal - 30 kg ha^{-1} , Maguisal - 10 kg ha^{-1} , Poly-feed – 25-40 kg ha^{-1}. Five treatments were tested: control treatment I0F0: 100% (ETc) - full irrigation and without fertigation; I1F1; I1F2; I2F1; I2F2. Irrigation was applied trough drip system include NMC Junior controller for precise irrigation rate application, FertiKit Nutrigation system for precise fertigation rate application, pressure-compensated pipelines UniRam AS with 14.6 mm inside diameter, 1.2 mm wall thickness, built-in trough 20 cm drippers and flow rate 1.6 l h^{-1}.

The microclimate data (air temperature, relative humidity, solar radiation, sunshine duration and wind speed) in the greenhouse was measured at every 30 min using an automatic meteorological station located in the center of experimental area and recorded in data logger (HOBO USB Micro Data Logger, USA). FAO Penman-Monteith Equation (Allen et al., 2006a) was used for determining daily reference evapotranspiration and irrigation scheduling. Crop coefficient was 0.30, 0.80 and 0.70 respectively in initial, middle and end growing stage (Allen et al., 2006b).

✓ **Methods**

Physicochemical parameters

- Total soluble solids by refractometer,% – BDS EN 12143-2000;
- Total soluble solids by weight, % – BDS 7133:1981;
- Moisture – BDS EN 12145:2000;
- Active acidity – BDS 11688-1993;

Biochemical indicators

- The antioxidant activity (DPPH-test) was determined according to the method of Brand- Williams et al. (1995) in the following modification: 2250 µl of DPPH solution (2.4 mg of DPPH in 100 ml of methanol) and 250 µl of sample extract.

A blank sample was similarly prepared using methanol instead of extract. After keeping the closed cuvettes in the dark for 15 min at a temperature of 20-25°C, the absorbance of the reaction mixture was measured at 515 nm.

The results obtained are presented as Trolox equivalents (GAE) per 100 g of extract.

- The content of total polyphenols in fruit and vegetable flour was determined according to the method of Singleton and Rossi (1965) with the following modification: 0.1 ml sample extract, ~7 ml distilled water, 0.5 ml Folin-Ciocalteu reagent (diluted 1:4 with distilled water) and 1.5 ml 7.5% (w/v) of aqueous sodium carbonate solution were successively dosed into a 10 ml measuring tube.

It was topped up to the mark with distilled water. After 2 hours at a temperature of 20-25°C, the absorbance of the reaction mixture was measured at 750 nm.

A blank sample was similarly prepared using distilled water instead of extract. The results are presented as gallic acid equivalents (GAE) per 100 g of extract.

The obtained data were statistically analyzed using ANOVA software, as well as Microsoft Excel and STATISTICA 8.0. Duncan's Multiple Range tests at a significance level ($p<0.05$) to measure specific differences between pairs of means was used.

RESULTS AND DISCUSSION

The results of the analyses conducted on the different growing variants of white strawberry fruits, based on harvests and storage duration, are presented in Figures 1, 2, and 3.

During the second vegetative period, the fruits from the three harvests across all irrigation and fertilization variants showed good quality results up to the 6th day of storage at 4°C.

Data from the physicochemical analyses indicate that, in terms of dry matter (refractometric), the soluble solids in the control group (I0F0 with 100% irrigation) increased to 9.13% during the second harvest, while the values for the first and third harvests were statistically indistinguishable ($p > 0.05$). The harvest factor had an effect on the control fruits ($p < 0.05$).

For the fruits from variant I1F1 (with 75% irrigation and 100% fertilization), the results were higher compared to those of the control (I0F0) only during the third harvest.

The percentage of soluble solids during the first and second harvests were statistically indistinguishable and lower than those of the control group I0F0 ($p > 0.05$). Here, the harvest factor had an effect only on the values from the third harvest ($p < 0.05$).

For the fruits from the third variant, I1F2 (with 75% irrigation and 75% fertilization), the percentage of soluble solids was the same as those in the control group, I0F0 (100% irrigation), and the second growing variant, I1F1 (75% irrigation and 100% fertilization) ($p > 0.05$). For these irrigation and fertigation variants, compared to the control I0F0 (100% irrigation), there were no statistically significant differences in soluble solids for the first harvest ($p > 0.05$). The timing of the harvests in this growing variant had a significant impact during the second and third harvests, with values being significantly lower during the second harvest (6.13%) compared to the first harvest, and increasing to 10.87% during the third harvest ($p < 0.05$).

For the fruits from the fourth (I2F1 with 50% irrigation and 100% fertilization) and fifth (I2F2 with 50% irrigation and 75% fertilization) growing variants, it was found that the percentage of soluble solids in the first harvest was lower than that of the control group, I0F0 (100% irrigation), and the above-mentioned variants, and statistically indistinguishable from each other, with values above 6% ($p > 0.05$). In this case, the growing method for the fruits from the first harvest of these two variants did not influence the measured values ($p > 0.05$). The selection of the percentage ratio of irrigation and fertilization for these two variants, compared to the control I0F0 (100% irrigation) and the variants I1F2 (with 75% irrigation and fertilization) and I1F1 (75% irrigation and 100% fertilization), had a significant impact on this parameter.

The timing of the harvests affects the second and third harvests, with dry matter content increasing gradually during the second harvest and reaching maximum values during the third harvest, at 12.5% and 11.43%, respectively, for each growing variant.

For all studied variants, depending on the harvests, the fruits from the third harvest in variant I1F1 (75% irrigation and 100% fertilization) had the highest soluble dry matter content at 12.67%, while the lowest content was 6.13% in the fruits from the first harvest of the fifth variant I2F2 (50% irrigation and 75% fertilization).

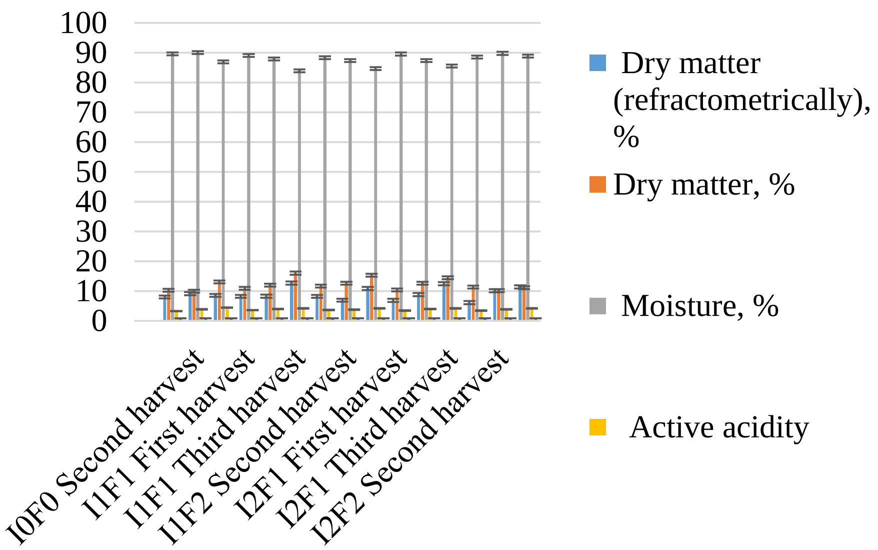


Figure 1. Physicochemical parameters of white strawberry fruits, depending on harvests and growing methods on the day of receipt

The timing and storage conditions influence the soluble dry matter values for fruits from the control group I0F0 (100% irrigation).

For the first harvest, the values are higher on the day of receipt compared to the three-day storage under refrigerated conditions, and are statistically indistinguishable from each other by the sixth day of storage ($p > 0.05$).

For fruits from the second harvest, storage conditions do not affect the percentage of measured dry matter on the day of receipt and during three-day storage ($p > 0.05$). A significant reduction is observed during six-day refrigerated storage compared to the day of receipt and the end of the storage period with other harvests, at 7.03% ($p < 0.05$).

The timing and storage conditions significantly affect soluble dry matter in fruits from the third harvest. During three-day storage, values are at their maximum for all harvests at 11%, and decrease by a factor of 1.1 by the sixth day of storage ($p < 0.05$). Only in fruits from the first harvest of the control group I0F0 (100% irrigation) is there an increase in soluble dry matter during six-day refrigerated storage compared to three-day storage.

In summary, soluble dry matter for the first harvest is statistically indistinguishable during six-day storage compared to the day of receipt, is the same for the second harvest up to the third day of storage, and decreases by the sixth day of storage, with an increase observed in fruits from the third harvest.

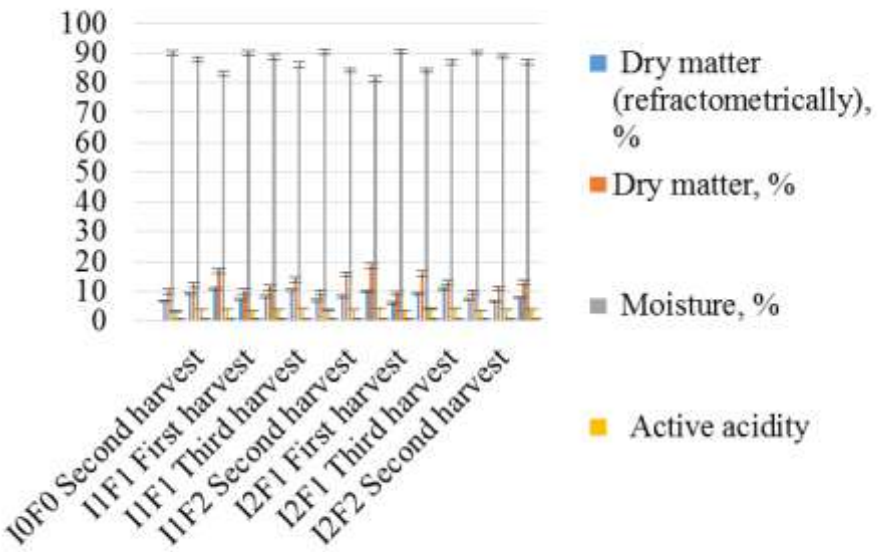


Figure 2. Physicochemical parameters of white strawberry fruits, depending on harvests and growing methods during three-day storage

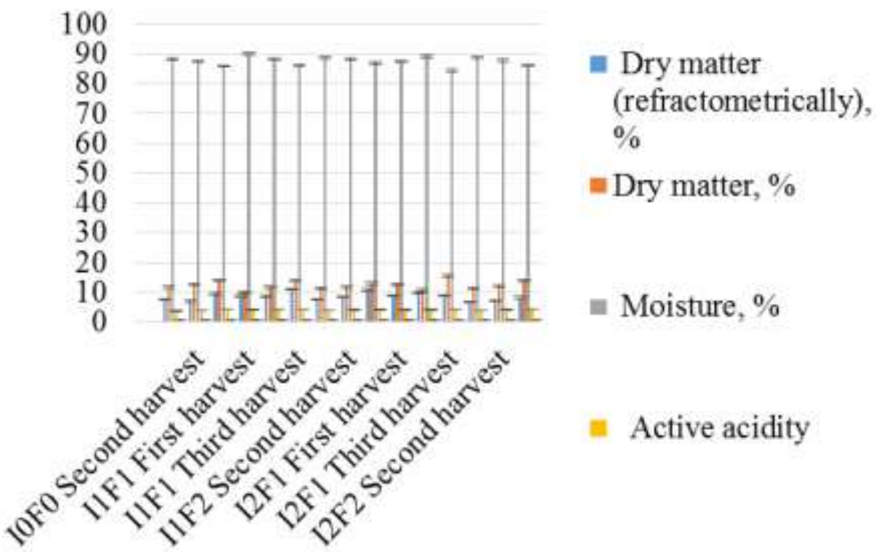


Figure 3. Physicochemical parameters of white strawberry fruits, depending on harvests and growing methods during six-day storage

Storage time affects the percentage values of soluble dry matter in fruits from the second variant I1F1 (with 75% irrigation and 100% fertilization) from the first and third harvests ($p < 0.05$). The timing of harvests and storage does not affect the values of soluble dry matter in fruits from the first and second harvests throughout the entire storage period ($p > 0.05$). The highest values are observed in fruits from the third harvest on the day of receipt at 12.67% and for the storage period at 11.07%, despite a reduction in values by a factor of 1.1.

For fruits from the third variant I1F2 (with equal irrigation and fertilization rates of 75%), harvests influence the values of soluble dry matter on the day of receipt, with the highest value recorded for fruits from the third harvest at 10.87%, which remains constant throughout the entire storage period ($p < 0.05$).

The time and storage conditions affect the first two studied harvests up to the third day of storage. For fruits from the first harvest, the percentage of dry matter decreases by a factor of 1, while for fruits from the second harvest, it increases by a factor of 1.2, reaching a value of 8.4% ($p < 0.05$). By the end of the storage period, the dry matter values (refractometric) remain unchanged for fruits from the first and third harvests ($p > 0.05$). Storage affects the values for this parameter in fruits from the second harvest ($p < 0.05$).

Harvests influence the soluble dry matter values in fruits from the fourth variant, I2F1 (50% irrigation and 100% fertilization), with the highest value on the day of receipt at 12.5%, and during three-day storage at 11% for fruits from the third harvest ($p < 0.05$). The lowest value is observed in fruits from the first harvest on the day of receipt at 6.88% and during three-day storage at 6.13%. The dry matter value remains unchanged for fruits from the second harvest at 9%. During the three-day storage, the soluble dry matter decreases by 1.5% for fruits from the first and third harvests. A significant reduction in values is measured at the end of the storage period for fruits from the third harvest, reaching 8.87%. For fruits from the first harvest, an increase in soluble dry matter is recorded at the end of the six-day refrigerated storage, similar to the increase observed for fruits from the third harvest, by a factor of 1.2. During the refrigerated storage period for this variant, regardless of the initial value on the day of harvest, the percentage of soluble dry matter for the three harvests is approximately equal at 9%.

In the last studied variant of white strawberry cultivation, I2F2 (50% irrigation and 75% fertilization), the timing of harvests affects the soluble dry matter values on the day of receipt ($p < 0.05$). The lowest value is 6.13% for fruits from the first harvest, while the highest value is 11.43% for fruits from the third harvest. During the three-day storage, only fruits from the first harvest show an increase in dry matter percentage to 7.35%, while fruits from the second and third harvests show a significant decrease, by factors of 1.6 and 1.5, respectively. At the end of the six-day storage, soluble dry matter values do not change for fruits from the second and third harvests, while for fruits from the first harvest, a decrease to 6.73% is observed.

Active acidity measured in fruits (depending on harvests and cultivation methods) on the day of receipt increases for all fruits from the third harvest and is above 4.1. The highest value is observed in fruits from the control group I0F0 (100% irrigation) from the third harvest at 4.47, while the other variants from this harvest with different irrigation and fertilization rates are statistically indistinguishable. Harvests influence this parameter, with the lowest values found in fruits from all variants of the first harvest, ranging from 3.27 in the control group I0F0 (100% irrigation) to 3.65 in fruits from the third variant I1F2 (with equal percentages of 75% irrigation and fertilization) ($p<0.05$). Cultivation method affects only the value in fruits from the control group I0F0 (100% irrigation) ($p<0.05$). Statistically indistinguishable active acidity values are found in fruits from the first harvest of variants with 75% irrigation and 100% fertilization, and 75% irrigation and 75% fertilization, as well as between variants with 50% irrigation and 100% fertilization and 50% irrigation and 75% fertilization. For these, the cultivation factor does not influence the active acidity values on the day of receipt ($p>0.05$).

Fruits from the second harvest have higher active acidity values compared to those from the first harvest, approaching 4, except for fruits from the second variant I1F2 (with 75% irrigation and 100% fertilization), whose values are not significantly different.

During the three-day refrigerated storage, the fruits from the first two harvests of all studied variants do not change their values compared to the day of receipt, and storage does not affect the investigated parameter ($p>0.05$). Harvest and storage period influence the reduction in active acidity values for fruits from the last fruiting period for variants from the control group I0F0 (100% irrigation) and I2F2 (50% irrigation and 75% fertilization) ($p<0.05$).

With minimal statistically indistinguishable values of active acidity after six-day storage (4.2), are fruits from the third harvest grown with 75% irrigation and 100% fertilization (I1F1) and 75% irrigation and 75% fertilization (I1F2) ($p>0.05$) (Figures 2 and 3). For all other cultivation variants and harvests of white strawberries, storage influences the active acidity, and values are statistically different, ranging from 3.77 in the control group I0F0 (100% irrigation) from the first harvest to 4.6 in fruits from the third harvest grown with 50% irrigation and 100% fertilization (I2F1) ($p<0.05$) (Figures 1, 2, and 3).

The summary for this parameter is that storage does not affect fruits from the third harvest, grown with 75% irrigation and 75% fertilization (I1F2) and with 75% irrigation and 100% fertilization (I1F1) ($p>0.05$). For all other variants from all harvests, storage influences the active acidity values of the fruits ($p<0.05$).

Moisture and dry matter (weight-based) in white strawberry fruits from the three harvests during storage were analyzed.

It was found that the moisture on the day of receipt for all studied cultivation variants depends on the harvests. The analyzed fruits from the first harvest have higher moisture percentages (approximately 90%) compared to other harvests, except for the I2F2 variant, which has the lowest percentages of irrigation and fertilization (50% irrigation and 75% fertilization). Statistical processing of the data showed no differences between values for fruits from the control group I0F0 (100% irrigation) and I2F1 (50% irrigation and 100% fertilization), as well as between I1F1 (75% irrigation and 100% fertilization), I1F2 (75% irrigation and 75% fertilization), and I2F2 (50% irrigation and 75% fertilization) variants for this harvest. Cultivation method does not affect this parameter ($p>0.05$).

For fruits from the second harvest, moisture values decrease by a factor of 1.1 compared to the first harvest for variants I1F1 (75% irrigation and 100% fertilization), I1F2 (75% irrigation and 75% fertilization), and I2F1 (50% irrigation and 100% fertilization), while values for variants I0F0 (100% irrigation) and I2F2 (50% irrigation and 75% fertilization) are statistically indistinguishable ($p>0.05$).

Fruits from the third harvest show a significant reduction in moisture percentage for variants I1F1 (75% irrigation and 100% fertilization), I1F2 (75% irrigation and 75% fertilization), and I2F1 (50% irrigation and 100% fertilization) (84%), with values being statistically indistinguishable from each other ($p>0.05$). The maximum moisture value is found in fruits from variant I2F2 (50% irrigation and 75% fertilization) from the third harvest (90.08%), while the minimum is in fruits from variant I1F1 (75% irrigation and 100% fertilization) from the third harvest (83.97%).

Storage influences the reduction in moisture content values for fruits grown in the control group I0F0 (100% irrigation) for all harvests, for variant I1F1 (75% irrigation and 100% fertilization) from the third harvest, I1F2 (75% irrigation and 75% fertilization) from the third harvest, and all harvests of fruits from variants I2F1 (50% irrigation and 100% fertilization) and I2F2 (50% irrigation and 75% fertilization).

For all other variants and harvests, storage does not influence the measured moisture ($p>0.05$) (Figures 1, 2, and 3). The maximum moisture percentage from all harvests and storage conditions is found in fruits grown with 75% irrigation and 100% fertilization from the first harvest (I1F1) (89.86%), while the lowest is in fruits grown with 50% irrigation and 100% fertilization (I2F1) from the third harvest (84.39%) ($p<0.05$).

Measured dry matter (weight-based) values for fruits from all harvests showed that harvests influence the recorded percentage values. Comparative analysis indicates that the highest values of insoluble dry matter are found in fruits from the third harvest, ranging from 11% for variant I2F1 (50% irrigation and 75% fertilization) to 16% for variant I1F1 (75% irrigation and 100% fertilization), compared to the first and second harvests with 10% for fruits from the control group I0F0 (100% irrigation) and I2F1 (50% irrigation and 75% fertilization) and 12.6% for fruits from I2F1 (50% irrigation and 75% fertilization) and I1F2 (75% irrigation and 75% fertilization).

Fruits from the first and second harvests for variants I0F0 (100% irrigation), I1F1 (75% irrigation and 100% fertilization), and I1F2 (75% irrigation and 75% fertilization) have no statistically significant differences, and their harvests do not affect the studied parameter. For fruits from variants I2F1 (50% irrigation and 100% fertilization), dry insoluble substances increase by a factor of 1.5, as well as for I2F2 (50% irrigation and 75% fertilization), with percentages decreasing to the same extent in the second harvest compared to the first.

Storage does not influence the values of insoluble dry matter for fruits from the control group I0F0 (100% irrigation) from the third harvest, I1F1 (75% irrigation and 100% fertilization) from the second harvest, and fruits from the first harvest of variants I1F2 (75% irrigation and 75% fertilization) and I2F2 (50% irrigation and 75% fertilization) ($p>0.05$) (Figures 1, 2, and 3).

For all other cultivation variants and harvests, storage conditions and duration influence the insoluble dry matter in fruits, with the highest average value after six-day storage being recorded for fruits from variant I2F1 (50% irrigation and 100% fertilization) from the third harvest (15.61%), and the lowest for fruits from variant I1F1 (75% irrigation and 100% fertilization) from the first harvest (10.14%) ($p<0.05$) (Figures 1, 2, and 3).

Biochemical indicators (antioxidant activity and total polyphenols) in fruits examined from all variants and harvests on the day of receipt showed that harvests have a significant impact on these indicators ($p<0.05$) (Figures 4 and 5).

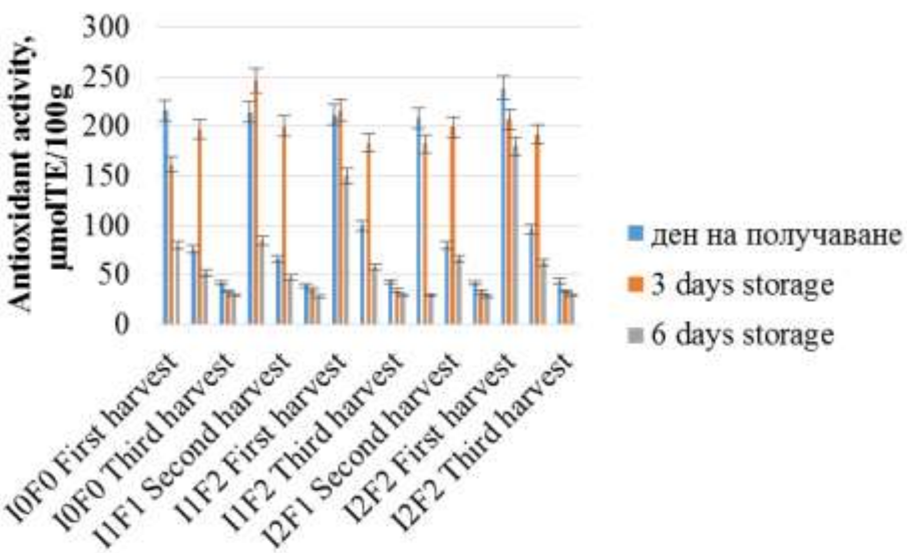


Figure 4. Antioxidant activity of white strawberry fruits depending on storage

On the day of receipt, fruits from all variants of the first harvest have significantly higher antioxidant activity values (over 200 µmolTE/100g) compared to the second harvest (over 70 µmolTE/100g) and the third harvest (over 40 µmolTE/100g) ($p<0.05$). The maximum antioxidant activity is found in fruits from the variant I2F2 (50% irrigation and 75% fertilization) from the third harvest at 238.87 µmolTE/100g, while the minimum is in fruits from the variant I1F1 (75% irrigation and 100% fertilization) from the third harvest at 39.3 µmolTE/100g (Figure 4).

It was determined that the applied agronomic conditions affect only the antioxidant activity in fruits from variant I2F2 (50% irrigation and 75% fertilization) for the first and second harvests, and in variant I1F2 (75% irrigation and 75% fertilization) for the second harvest ($p<0.05$) (Figure 4). For all other variants and harvests, the cultivation method does not influence the antioxidant activity values of the fruits ($p>0.05$) (Figure 4).

The duration and conditions of storage have a significant impact on the antioxidant activity of all fruits in the conducted test ($p<0.05$) (Figure 4).

During the three-day refrigerated storage of fruits from all variants and harvests, it was found that antioxidant activity decreases by approximately 1.5 times in fruits from the control group I0F0 (100% irrigation) from the first harvest, down to 161.3 µmolTE/100g, and by 1 order of magnitude in all fruits from the three harvests for the studied cultivation variants.

Fruits from variant I1F2 (75% irrigation and 75% fertilization) show relatively stable antioxidant activity up to this storage day, at 216.2 µmolTE/100g for the first harvest, and fruits from the same harvest of variant I2F1 (50% irrigation and 100% fertilization) at 182.5 µmolTE/100g.

At the end of the storage period, antioxidant activity significantly decreases by nearly 2-2.5 times in all examined variants compared to the day of receipt, with the lowest values in fruits from all variants of the third harvest (28.65 µmolTE/100g) for variants I1F1 (75% irrigation and 100% fertilization) and I2F1 (50% irrigation and 100% fertilization), and the highest in fruits from the first harvest of variant I2F2 (50% irrigation and 75% fertilization) at 180 µmolTE/100g.

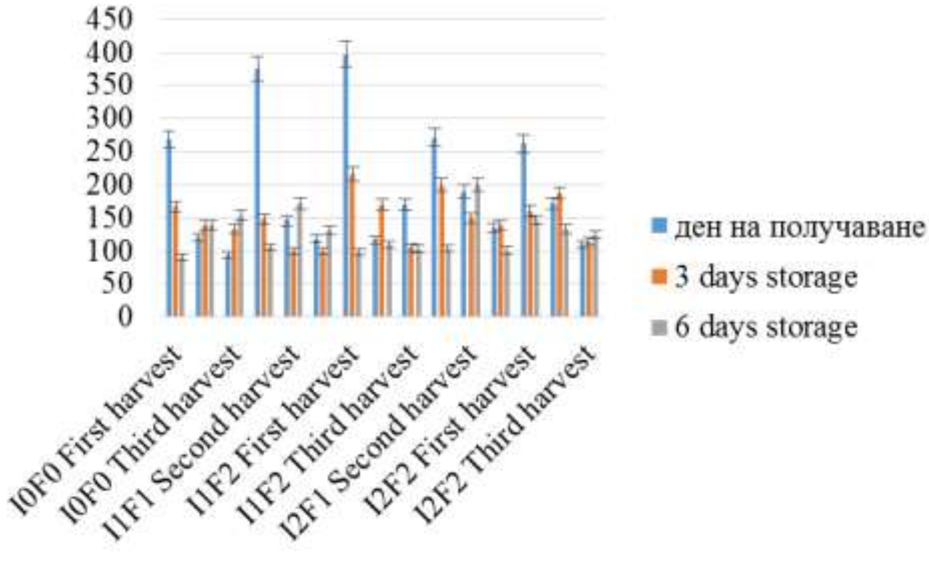


Figure 5. Total polyphenols in white strawberry fruits depending on storage

The total polyphenol content in fruits for all variants and harvests on the day of receipt showed that harvests influence the measured values ($p<0.05$) (Figure 5). Comparative analysis reveals that fruits from the first harvest for all studied variants have the highest total polyphenol values (over 260 mg GAE/100g) compared to those from the second harvest (100 mg GAE/100g) and the third harvest (90 mg GAE/100g).

The lowest values for the studied parameter are found in fruits from the third harvest, except for fruits from the variant I1F2 (75% irrigation and 75% fertilization). Statistically similar values of total polyphenols are found in fruits from the variants of the first harvest I1F1 (75% irrigation and 100% fertilization) and I1F2 (75% irrigation and 75% fertilization), as well as between I0F0 (100% irrigation), I2F1 (50% irrigation and 100% fertilization), and I2F2 (50% irrigation and 75% fertilization). For these variants, the cultivation method does not affect the total polyphenol content of the fruits ($p>0.05$) (Figure 5).

Temperature (T=4°C) and storage duration do not affect the total polyphenols in fruits from variants I2F2 (50% irrigation and 75% fertilization) and I1F1 (75% irrigation and 100% fertilization) from the third harvest, whose values at the end of the six-day storage are 124.00 mg GAE/100g and 131.00 mg GAE/100g, respectively.

For all other fruits from the studied variants and harvests, storage has an impact, with increases in total polyphenol content during storage in fruits from the control variant I0F0 (100% irrigation) for the second harvest (138.00 mg GAE/100g) and third harvest (153.00 mg GAE/100g), and in fruits from variant I1F1 (75% irrigation and 100% fertilization) for the second harvest (172.00 mg GAE/100g). In other cultivation variants and harvests, total polyphenol content decreases during storage, with the highest value at 200.00 mg GAE/100g in fruits from 50% irrigation and 100% fertilization during the second harvest, and the lowest value at 90.00 mg GAE/100g in fruits from the control variant of the first harvest. Storage duration affects the values of this parameter ($p<0.05$).

CONCLUSIONS

The impact of harvests, applied agronomic techniques, and storage time (at 4°C for 6 days) on the quality characteristics of white strawberry (Fragaria x Ananassa "Snow White") fruits has been investigated. It was found that storage affects the physicochemical and biochemical parameters of the studied fruits ($p<0.05$).

Storage time and conditions have a significant impact on the antioxidant activity of all fruits tested ($p<0.05$). At the end of the storage period, antioxidant activity significantly decreases by about 2-2.5 times in all studied variants compared to the day of receipt, with the lowest values found in fruits from all variants of the third harvest (28.65 µmolTE/100g) from variants I1F1 (75% irrigation and 100% fertilization) and I2F1 (50% irrigation and 100% fertilization), while the highest values are observed in fruits from the first harvest with variant I2F2 (50% irrigation and 75% fertilization) at 180 µmolTE/100g.

Temperature (T=4°C) and storage duration do not affect the total polyphenol content in fruits from variants I2F2 (50% irrigation and 75% fertilization) and I1F1 (75% irrigation and 100% fertilization) from the third harvest. For all other variants of the biochemical tests conducted, storage has an impact ($p<0.05$).

ACKNOWLEDGEMENTS

This research work was carried out with the financial support of the BULGARIAN NATIONAL SCIENCE FUND „Competition for financial support for basic research projects – 2022", project title "Impact of the soil and weather parameters on the irrigation management and elaboration of biological means to control phytopathogens to enhance strawberry fruit quality" (grant No KП-06-H66/4).

The authors also want to thank *Haifa South East Europe* for providing whole range of water soluble fertilizers for conducting the experiments.

REFERENCES

Allen, R. G., L. S. Pereira, D. Raes, M. Smith. 2006a. Crop evapotranspiration (guidelines for computing crop water requirements). FAO Irrigation and drainage paper 56. (Food and Agriculture Organization of the United Nations, Rome), pp.24.

Allen, R. G., L. S. Pereira, D. Raes, M. Smith. 2006b. Crop evapotranspiration (guidelines for computing crop water requirements). FAO Irrigation and drainage paper 56. (Food and Agriculture Organization of the United Nations, Rome), pp.138.

Balan, H., A. Melnychenko, V. Sergiyenko, Y. Enakiev, D. Domuschi. 2024. Improvement of technology in the cultivation of strawberries hybrid Soraya F1 during a vegetative trial. BIO Web of Conferences 122, 01024 EE&AE-2024, 1-13.

Brand-Williams, W., M. Cuvelier and C.Berset, 1995. Use of a Free Radical method to evaluate antioxidant activity. Food Science and Technology- LebensmittelWissenschaft and Technologie, 28 (1), 25–30.

Singleton, V and J. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 16, 144–158.

Cordenunsi, B.R., J.R.O. Nascimento, F.M. Lajolo. 2003. Physico-chemical changes related to quality of five strawberry fruit cultivars during cool-storage, Food Chemistry. 83 (2), 167-173. https://doi.org/10.1016/S0308-8146(03)00059-1

Cirico T.L, S.T. Omaye. 2006. Additive or synergetic effects of phenolic compounds on human low density lipoprotein oxidation. Food and Chemical Toxicology, 44(4):510-6. https://doi.org/10.1016/j.fct.2005.08.025

Galli, R. L., D. F. Bielinski, A. Szprengiel, B. Shukitt-Hale, J.A. Joseph.., 2006. The blueberry-supplemented diet reverses the age-related decline in hippocampal HSP70 neuroprotection. Neurobio aging, 27, 344–350.

Haifa Group. (2021). https://www.haifa-group.com/crop-guide/vegetables/strawberry-fertilizer/crop-guide-strawberry-fertilizer-recommendations

Ivanova P., E. Grancharova, B. Elenov. 2024. Influence of irrigation and fertigation on physico-chemical and biochemical characteristics of fruits of white strawberry (*Fragaria x Anannassa "Snow White"*) grown in Bulgaria. Journal of Mountain Agriculture on the Balkans, 27 (1): 201–227.

Karakaya, S., 2004. Bioavailability of phenolic compounds, Crit. Rev. Food Sci. Nutr. 44, 453–464.

Kumar, S., S. Prakash, V. Kumar, A. Kumar. 2021. Varietal evaluation of the strawberry (*Fragaria × ananassa Duch*) based on the yield and physico-chemical parameters. Indian Journal of Plant Genetic Resources, 34(1):54-58. https://doi.org/10.5958/0976-1926.2021.00007.3

Markovic, J.M.D., N.A. Petranovic, J.M. Baranac. 2000. A spectrophotometric study of the copigmentation of malvin with caffeic and ferulic acids. J. Agric. Food Chem. 48, 5530-5536.

Moyer, R., K. Hammer, S. Finn, B. Frei, R. Wrolstad. 2002. Anthocyanins, phenols and antioxidant capacity in various small fruits: Vaccinium, Rubus and Ribes. Journal of Agricultural and Food Chemistry 50: 519–525.

Olbricht, K., U. Gerischer, A. Ludwig, D. Ulrich, B. Casas, A. Darbonne, S. Kraege, H. Obers, A. Vissers, P. Walpole, S. Walpole. 2013. "Snow White", a new strawberry cultivar with white fruits. Book of abstracts Growing strawberries in the next decade: intonation, the key of success? International Strawberry congress, Belgium. Retrieved July 1 2021, from https://iris.unipa.it/retrieve/handle/10447/99579/128625/Book%20of%20abstracts%20ISC2013.pdf

Pinheiro, D. F., J. T. Resende, L. V. Constantino, F. T. Hata, N. N. Y. Hata, S. B. Lustosa. 2021. Physical, biochemical, and sensory properties of strawberries grown in high-altitude tropical climate. Ciência e Agrotecnologia, 45:e008221. http://dx.doi.org/10.1590/1413-7054202145008221

Ramirez-Tortosa, C., O. M Anderson, P. T. Gardner, P. C. Morrice. 2001. Anthocyanin-rich extract decreases indices of lipid peroxidation and DNA damage in vitamin E-depleted rats, Free Rad. Biol. Med., 31, 1033–1037.

Sadik, H. Ch. Ouazzani, A. Moustaghfir, A. Er-Ramly, S. El Ghammarti, A. Touzani, A. Dami, L. Ballouch. 2023. Study of the physicochemical characteristics of different strawberries consumed in Morocco. Current research in nutrition and food science, 11(1), 339-350. http://dx.doi.org/10.12944/CRNFSJ.11.1.25

Wigati, L. P., A. A. Wardana, J. S. Jothi, S. Leonard, T. T. Van, X. Yan, F. Tanaka, F. Tanaka. 2024. Biochemical and color stability preservation of strawberry using edible coatings based on jicama starch/calcium propionate/agarwood bouya essential oil during cold storage. Journal of Stored Products Research, 107, 102324. https://doi.org/10.1016/j.jspr.2024.102324

THE EFFECTS OF CHITOSAN AND ESSENTIAL OIL APPLICATIONS ON FRUIT CRACKING PREVENTION AND QUALITY CRITERIA IN 0900 ZIRAAT CHERRY VARIETY (*PRUNUS AVIUM.* L)

Burhanettin Imrak[1]Eren AKKAYA[1], Songül ÇÖMLEKÇIOGLU[1]

[1] Cukurova University, Agriculture Faculty, Horticulture Department Adana, Turkey
Corresponding author e-mail: imrakburhan@gmail.com

ABSTRACT

This study was conducted during the 2022-2023 period in the experimental plot located at the Çukurova University Pozantı Agricultural Research and Application Center, situated at an altitude of 1200 m. The study focused on the 0900 Ziraat cherry variety grafted onto MaxMa 60 rootstock, planted at a 5x5m spacing, and pruned in a Central Leader system. The trees were five years old. In the study, %0.5 $CaCl_2$, 300 ppm Green Stim, 100 ppm Chitosan, 400 ppm Thyme oil, combinations of these treatments, and water as a control were used. The study was conducted in a randomized complete block design with 3 replications, each containing 10 plants. As a result of this study, while the control application showed a 36% cracking rate, the lowest fruit cracking rate of 2% was obtained with the Thyme Oil (400 ppm) application. Particularly, the Thyme oil application had positive effects on fruit size. The fruit weight, which was 7.39 g in the control, was determined to be 8.42 g with the Thyme oil application, indicating a positive effect on fruit weight. The best result in fruit firmness was also achieved with the Thyme oil application. While the control had a fruit firmness of 4.5 kg, the Thyme oil application resulted in a fruit firmness of 5.42 kg.

Keywords: Cherry, quality, fruit cracking, essential oil

INTRODUCTION

Cherry (Prunus avium) is a type of stone fruit from the Rosaceae family, native to Asia (Özbek, 1978; Webster et al., 1996). Fruits, which constitute an important group among the species belonging to horticultural plants, are of great importance with their production values, growing areas, nutrition and share in exports. Fruits and vegetables are a source of vitamins, minerals, dietary fiber and antioxidants in terms of nutrition. The ratio of water-soluble dry matter, skin color, hardness of the fruit flesh, fruit diameter and weight are the most important criteria affecting consumer preferences in cherry, which is a low-calorie, good source of calcium, potassium and vitamins (vitamins A, B1, B2, C and malic acid) (Wani et al., 2014).

Cherries are produced in a wide area around the world. However, they are mostly produced in Turkey, the USA, Iran and Italy in commercial terms. Turkey, which ranks first in cherry production with the changes in climate conditions and years, is also one of the countries that have a say in cherry exports. Turkey's geographical structure and climate conditions allow for high-quality cherry cultivation suitable for export. The harvest time of cherries grown in Turkey can be earlier than in many European countries, and it extends until August as they rise above sea level (Küden, 2004). Cherries are grown almost everywhere in our country. Cherry production in our country is mainly carried out in the Kemalpaşa district of İzmir, Manisa, Konya-Akşehir, Afyonkarahisar-Sultandağı, Isparta-Uluborlu, Denizli-Honaz and recently in the Konya Hadim and Taşkent regions. It is also produced in Çanakkale, Kütahya, Mersin, Antalya, Kahramanmaraş, Adana (Saimbeyli), Niğde (Ulukışla), Bursa, Amasya and Sakarya.

The area of cherry orchards in 2023 is 798,622 decares (Figure 1), export value and volume (Figure 2), the production amount is 736,792 tons (TÜİK, 2024).

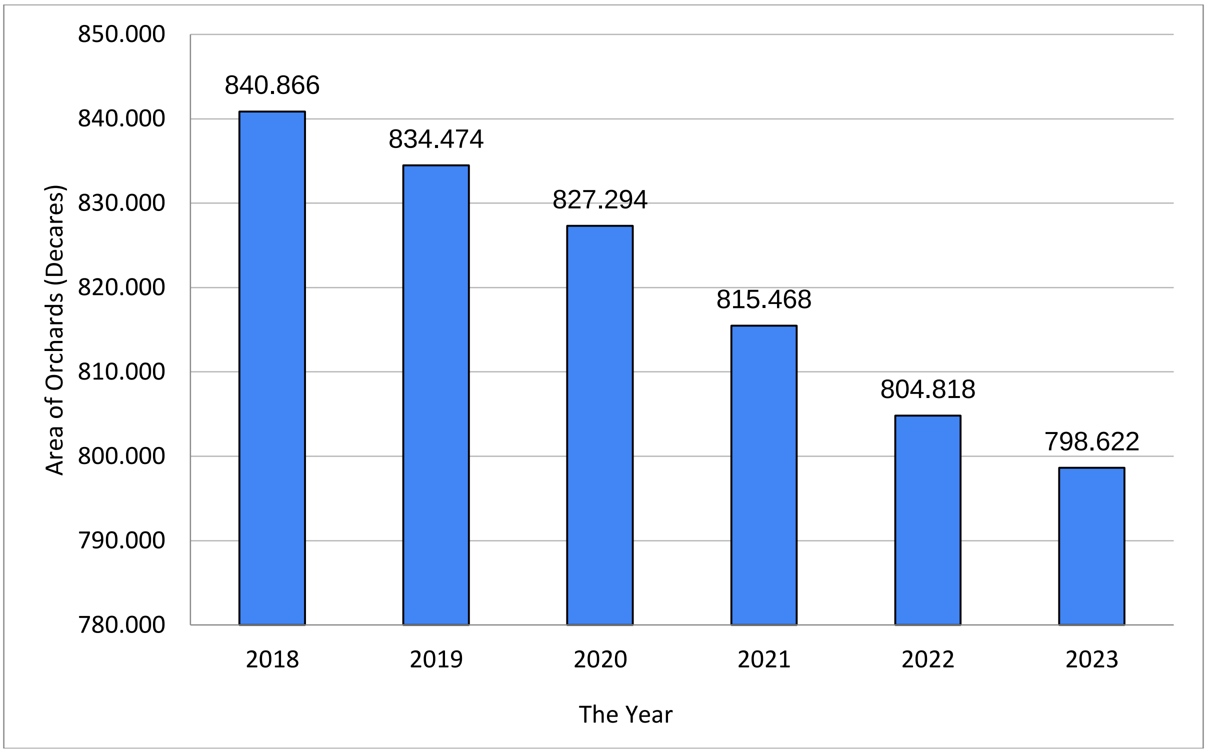


Figure 1. Turkey’s Cherry production area by years

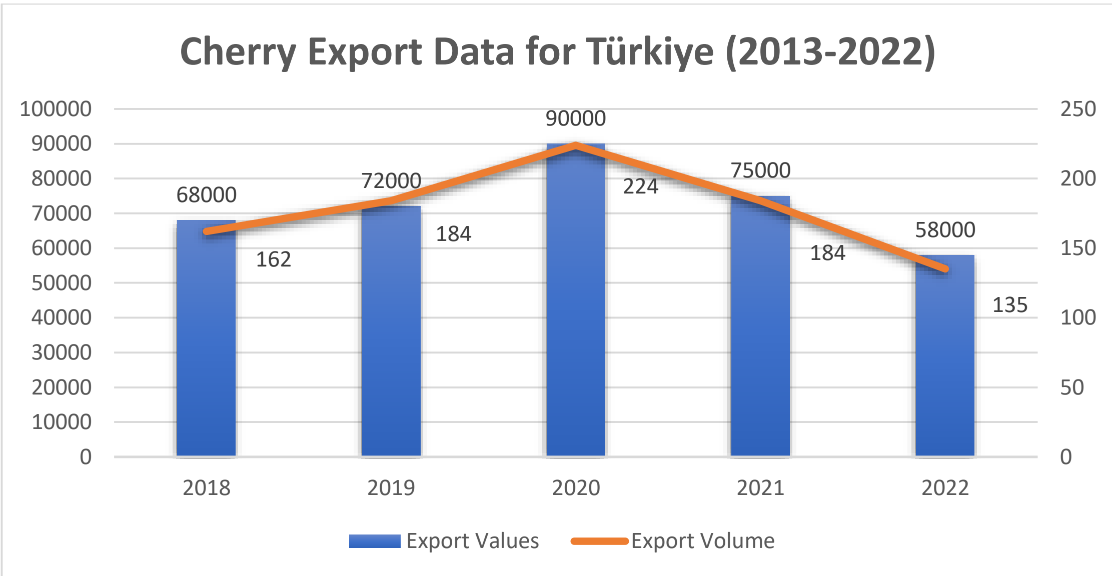


Figure 2. Turkey’s Cherry export values and volume by years

One of the most important problems arising from climatic events in cherry cultivation is fruit cracking due to excessive rainfall occurring during harvest time and close to harvest, which causes great economic losses (İmrak et al., 2018; İmrak and Küden 2023). Cracking in cherries occurs in the cuticle of the fruit and generally occurs at 3 points (stalk pit, lateral face, and fruit tip) (Peschel and Knoche, 2005). The most common cause of cracking is accepted as rainwater entering the fruit shell (Davenport, 1972). Although research on the causes of cracking began in the 1930s, this issue is still debated among scientists (Sekse, 1995). It has been accepted that many factors such as soil moisture, tree-water relations (Sekse, 1995), variety (Christensen, 1972a), fruit maturity stage (Christensen, 1973), soluble substance concentration (Christensen, 1972b), temperature (Christensen, 1972c), stomatal width and frequency (Christensen, 1972b), respiration rate (Pommier, 1989) play an important role in the cracking event.

In the study examining the susceptibility of Fermina, Regina, Lapins and ‘Brooks varieties to cracking, it was stated that Regina and Fermina varieties were more resistant than Lapins and Brooks varieties (Moing et al., 2004). It is known that Regina variety is one of the most resistant varieties to fruit cracking (İmrak et al., 2018).

The main purpose taken into consideration in the selection of chemicals applied to reduce cracking is to reduce the amount of rainwater entering the fruit surface as much as possible during and after heavy rain. In many areas, calcium-containing solutions and the use of calcium together with substances such as copper, sulfate, nitrate are used against fruit cracking, as well as antitranspirants and various surface waxes (Sekse, 1995).

In cherry cultivation, calcium-containing solutions, which are used intensively to reduce cracking, are used effectively in many areas 2-3 weeks before harvest (Brown et al., 1995). In calcium applications, calcium blocks cell wall pectins, making the cell wall resistant to pressure and cracking (Alani, 1980).

In recent years, many applications have been made to prevent cracking, and one of them is the application of 0.5 mM methyl jasmonate applied in the pre-harvest period, which is stated to have a positive effect in preventing fruit cracking by increasing tolerance to abiotic stress (Ruiz-Aracil et al., 2023)

In the research, it was aimed to prevent cracking in the fruits of 0900 Ziraat cherry variety for export, caused by excessive rainfall during harvest time, by applying Calcium Chloride ($CaCl_2$), Green Stim, Chitosan and Thyme oil, thus increasing the quantity and quality of fruit for export.

MATERIAL AND METHOD

This study was carried out on 5-year-old 0900 Ziraat cherry variety grafted onto MaxMa 60 rootstock, planted at 5x5m planting distance and pruned in the Central Leader system in the experimental area located at Çukurova University Pozantı Agricultural Research and Application Center at an altitude of 1200m in the 2022-2023 period. In the study; 0.5% $CaCl_2$, 300ppm Green Stim, 100ppm Chitosan, 400ppm Thyme oil and water were used as control. The study was carried out according to the randomized block trial design with 3 replications and 10 plants in each replication.

Plant Material

0900 Ziraat cherry variety, which is mid resistant to cracking, is known as Turkish Cherry in Europe. Fruit characteristics; colour is bright dark red and large and heart-shaped fruits are resistant to transportation and storage. It has a thin, long and green stem, and its flesh is hard, juicy and delicious; with these features, it is among the best cherries in the world (Demirtaş and Sarısu, 2011).

It is in the Bigarreau group. It ripens late. Its fruit is very large (8.15 g), wide heart-shaped and bright dark red, fruit flesh is quite hard, crisp, juicy and very delicious; quality is excellent but needs pollinator, resistant to bacterial cancer and its flowering time is quite late. Its net mesocarp ratio is 14.31 and it is resistant to transport (Anonymous 2010).

Applications to Prevent Cracking in Fruits and Increase Quality

The plant growth regulators used in the study are given below. Water application was used as a control.

1- Calcium chloride ($CaCl_2$)
2- Calcium chloride ($CaCl_2$) + Green Stim
3- Calcium chloride ($CaCl_2$) + Chitosan
4- Calcium chloride ($CaCl_2$) + Thyme oil

Calcium chloride (CaCl2)

It is an inorganic compound, a salt with the chemical formula $CaCl_2$. It is a white crystalline solid at room temperature and is quite soluble in water. It can be formed by neutralizing hydrochloric acid with calcium hydroxide. Calcium chloride is commonly found as a hydrated solid with $CaCl_2 \cdot n\ H_2O$. These compounds are mainly used for deicing and dust control. Since anhydrous salt is hydroscopic and liquid, it is used as a desiccant (Kemp and Keegan, 2000). In calcium applications, calcium makes the cell wall resistant to pressure and cracking as a result of blocking cell wall pectin's (Alani, 1980).

The main purpose taken into consideration in the selection of chemicals applied to reduce cracking is to reduce the amount of rainwater entering the fruit surface as much as possible during and after heavy rain. In many areas, calcium-containing solutions and the use of calcium together with substances such as copper, sulfate, nitrate, as well as antitranspirants and various surface waxes are used against fruit cracking (Sekse, 1995).

Green Stim

It contains natural osmotic pressure balancers (glycine, betaine) (Imrak, 2019).

Chitosan

Chitosan is obtained as a result of deacetylation of chitin in an alkaline environment. It is insoluble in water and organic-based solvents (Abdou et al., 2008). Chitosan, the most widely used polysaccharide, is an economical natural biopolymer obtained from sea shells. (Sandford, 1989).

Thyme Oil

In our country, there are many fragrant plant species known as "Thyme" belonging to the Lamiaceae family, and their essential oils especially contain carvacrol and thymol. Studies have determined that Thyme oil suppresses many fungal diseases (Baydar, 2007; Bosquez et al., 2010; Camele et al., 2012).

General Characteristics of the Research Area

The research was carried out in the experimental area of Ç.Ü. Pozantı Agricultural Research Center in Pozantı, which is 1200 meters above sea level. Coordinate: 37.480050,34.903235.

The study was conducted between 2022-2023 and was set up in a randomized plot design with 3 replications and 10 trees in each replication. All applications were made approximately 30 days before harvest when the fruits turned from yellow to pink (vein fall) with a 15-liter backpack pump to wet the entire tree. The applications and their doses are given in detail in Table 1. All spray treatments were applied when the fruit color turns from green to yellow (22.06.2022)

Table 1. Applications and doses

Applications	Doses
Control	Water
$CaCl_2$	%0.5
$CaCl_2$ + Chitosan	%0.5 $CaCl_2$ + Chitosan 100 ppm
$CaCl_2$ + Thyme Oil	%0.5 $CaCl_2$ + Thyme Oil 400 ppm
$CaCl_2$ + Green Stim	%0.5 $CaCl_2$ + Green Stim 300 ppm

Phenological Observations

Budbreak, full bloom and harvesting dates were recorded (Westwood, 1987).

Pomological Analyses

Pomological analyses were performed with 3 repetitions and on 10 fruits at harvest maturity in each repetition (Emerce, 2004; İmrak, 2019).

Average Fruit Weight (g): The fruits obtained were weighed with a scale with a sensitivity of 0.01 g and calculated by dividing the total weight by the number of fruits. Here, 30 randomly taken fruit samples that could represent the analyzed variety were divided into 3, with 10 fruits in each repetition, and these fruits were weighed individually to determine the average fruit weight of each repetition.

The fruits obtained were measured with a caliper with a sensitivity of 0.01 mm and calculated by dividing the total value by the number of fruits. fruit length (mm), fruit width (mm), fruit height (mm)

Total Soluble Solids (TSS) %: The percentage value of the samples taken from the fruit juice was measured with a hand refractometer.

Total Acidity (%): After completing 5 ml of fruit juice with distilled pure water to 100 ml, titratable 0.1 Normality NaOH (Sodium Hydroxide) will be added to 5 ml of fruit juice completed with distilled water to 100 ml with the help of a pH meter until the digital display on the pH meter reaches 8.00-8.10 and then calculated in terms of citric acid according to the formula below.

Acidity Formula = Amount of NaOH consumed x 20 x Factor (0.963) x Acid value (Acid value for citric acid, 0.0067).

Fruit Flesh Firmness (kg): The hardness of the fruits of the varieties in the trial was measured using a hand penetrometer.

Color Measurements

Color measurements made according to the C.I.E. L*a*b* system were carried out with a Minolta color measuring device. In this system, the a* value indicates the color change from green to red, and the b* value indicates the color change from blue to yellow. Positive values of a* indicate red, and negative values indicate green. Positive values of b* indicate yellow, and negative values indicate blue (İmrak et al., 2018). The data obtained from the experiment were analyzed using the JMP v.8 statistical package program and the averages were compared using the Tukey test.

Cracking Index

The cracking index will be calculated in order to determine the cracking prevention or reduction effects of the applications (Bilginer et al., 1999). In this method, cherries that reached harvest maturity were collected in the morning hours (09:00-11:00) and brought to the laboratory for analysis in the cold chain.

In order to measure the cracking resistance of cherries, 50 fruits randomly selected from the fruits located 1.5 m above the ground in the outward facing area of the trees were used. In this method, cherries that reached harvest maturity were collected before noon and brought to the laboratory for analysis in the cold chain. In order to measure the resistance of cherries to cracking, 50 randomly selected fruits were placed in 2-liter glass jars and kept in pure water at 20oC for 2-4-6 hours. At 2-hour intervals, the fruits were removed from the water and the cracked fruits were counted, and the healthy fruits were put back into the water. The same procedures were performed after 4 and 6 hours. The obtained data were calculated according to the cracking index formula used by Bilginer et al (1999) for cherries and explained below.

Cracking index = (5a + 3b + c) x 100/250

Statistical Analysis

The experiment was based on randomized blocking pattern as 3 replications and was assigned as one tree at each replication. The data were statistically analyzed using the Statistical Package for the. Means separation was determined by LSD test. Statistical analyses were carried out using JMP 5.0.1 version.

RESULTS AND DISCUSSION

It was observed that the applications made did not have any effect on the phenological stages. Phenological periods are presented in Table 2. It was determined that the dates specified in terms of phenological periods were parallel to previous studies and were found to be compatible with (İmrak and Küden, 2023).

Table 2. The effects of Applications on phenological stages in ‘0900 Ziraat’ sweet cherry variety

Variety	Dormancy Breaking	Full Bloom	Harvesting time
0900 Ziraat	15.02.2023	19.04.2023	21.06.2023

Among the applications applied to the 0900 Ziraat cherry variety included in the experiment, the highest fruit flesh hardness was determined as 5.42 kg in the Thyme oil application, while the lowest fruit flesh hardness was determined as 4.5 kg in the control application. The fruit flesh hardness obtained as a result of other applications is between these values. Thyme oil application provided the highest fruit flesh hardness and clearly distinguished itself from all other applications. Thyme oil is known to have strong antimicrobial and antifungal properties among vegetable oils and can provide a protective effect on fruit tissue. This result supports the effectiveness of thyme oil in increasing fruit quality. There are findings in the literature that thyme oil applications provide similar positive effects in other fruit species (Sivakumar and Bautista-Baños, 2014). Chitosan application also showed a significant effect on fruit flesh hardness. With 4.83 kg fruit flesh hardness, chitosan application provided a significant increase compared to the control group. Chitosan is a natural biopolymer that can prevent water loss and microbial infections by forming a protective layer on the fruit surface. This shows that chitosan is an effective agent in improving fruit quality. Similarly, chitosan applications have been reported to increase fruit flesh firmness in other fruit species (El Ghaouth et al., 1992; Bautista-Baños et al., 2006). Calcium chloride application increased fruit flesh firmness to 4.65 kg. Calcium may increase fruit firmness by contributing to the strengthening of cell walls and the increase in the water retention capacity of cells. This finding is consistent with the literature showing that calcium applications provide similar effects in other fruit species (Conway et al., 1994; Saure, 2005) (Table 3).

The lowest L* value found in color measurement was determined to be 19.61 for Thyme oil application, while the highest L* value was determined to be 26.78 for Chitosan application (Table 3). The lowest a* value was determined to be 14.03 for Thyme oil application, while the highest a* value was determined to be 23.96 for control application. In addition, the lowest b* value was determined to be 5.06 for Thyme oil application, while the highest b* value was determined to be 9.23 for control application.

Table 3. The effects of Applications on fruit frimness and colour characteristics in '0900 Ziraat' sweet cherry variety

Applications	**Fruit Frimness (kg)**	**L***	**a***	**b***
Control	4,5d	23,29ab	23,96a	7,01
$CaCl_2$	4,65c	20,44ab	17,21ab	6,06
$CaCl_2$+ Chitosan	4,83b	26,78a	17,38ab	6,47
$CaCl_2$ + Green Stim	4,53bc	24,8b	17,39ab	6,07
$CaCl_2$ + Thyme Oil	5,42a	19,61b	14,03b	5,06
$Lsd_{\%5}$	0,20	5,47	5,42	ns

Among the applications applied to the 0900 Ziraat cherry variety in the trial, the highest fruit weight was determined as 8.42 g in the Thyme Oil application, while the lowest fruit weight was determined as 7.39 g in the control. When we look at the average fruit weight data of all applications and control groups, it was determined that the average fruit weight of the Thyme Oil was higher (Table 4). The lowest SÇKM amount measurement value of the applications was measured as 14.63 in Green Stim, while the highest value was determined as 15.33 in Chitosan. In the pH value measurements of the applications in the trial, the lowest value was measured in the Calcium application as 2.88. The highest value was measured in Green Stim as 2.94. It was determined that the pH value of all applications and control groups was the highest and the lowest was in the Calcium application.

The Calcium application provided the lowest pH value (2.88) and was significantly different from all other applications and the control group. The potential of calcium to reduce fruit pH may be due to the interaction of calcium ions with cell walls and intracellular matrices and increasing acidic properties. Similar findings have been reported in the literature that calcium applications improve product quality by reducing fruit pH (Conway et al., 1994; Saure, 2005).

The control group and chitosan application showed similar results with a pH value of 2.92. Chitosan can maintain pH balance by forming a protective layer on the fruit surface with its biopolymer structure. There are findings in the literature that chitosan improves fruit quality and provides pH balance (El Ghaouth et al., 1992; Bautista-Banos et al., 2006). Oregano oil application showed a result close to other applications with a pH value of 2.93. The antimicrobial and antifungal properties of oregano oil can help maintain pH balance by reducing microbial activity on the fruit surface. Similar findings on the positive effects of oregano oil applications on fruit quality are also available in the literature (Sivakumar and Bautista-Banos, 2014) (Table 4).

Table 4. The effects of Applications on pomological characteristics in '0900 Ziraat' sweet cherry variety

Applications	Average fruit weight (g)	Total soluble solid content TSS (%)	Titrable acidity (TA) (g citric acid/100 ml)
Control	7,39b	14,93	2,92ab
$CaCl_2$	7,97ab	15,23	2,88b
$CaCl_2$+ Chitosan	7,89ab	15,33	2,92ab
$CaCl_2$ + Green Stim	7,97ab	14,63	2,94a
$CaCl_2$ + Thyme Oil	8,42a	15,17	2,93ab
$Lsd_{\%5}$	0,77	Ns	0,09

In the control group, the fruit cracking rate was determined to be the highest at 11.2%. This shows that a significant portion of the cherry fruits cracked when no application was made. 0.5% Calcium Chloride ($CaCl_2$) application reduced the fruit cracking rate to 2.4%. This shows that $CaCl_2$ significantly reduces fruit cracking. 300 ppm Green Stim application reduced the fruit cracking rate to 0.8%. This shows that Green Stim greatly reduces fruit cracking and is an effective application. 100 ppm Chitosan application also reduced the fruit cracking rate to 0.8%. This shows that chitosan is effective in reducing fruit cracking. 400 ppm Thyme Oil application achieved the lowest fruit cracking rate of 0.4%. This shows that thyme oil is the most effective application in preventing fruit cracking (Figure 3)

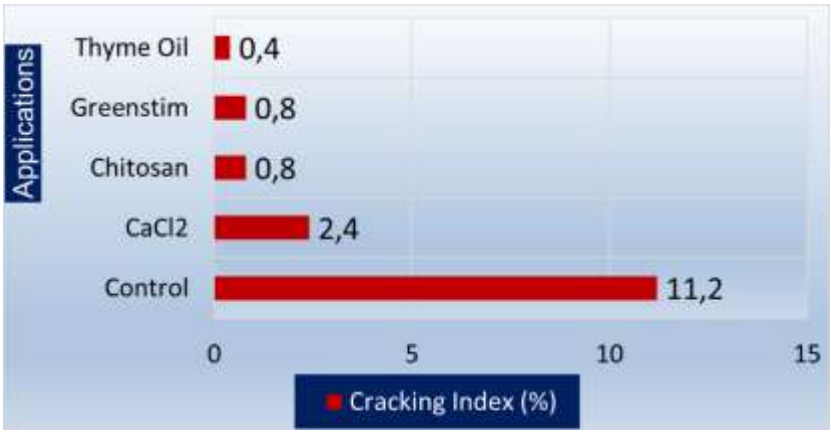


Figure 3. Effects of different treatments on 0900 Ziraat cherry fruit cracking Index

CONCLUSIONS

The results of this study have shown that all treatments significantly decreased the cracked fruit ratio compared to the control. But Thyme oil applications especially reduced the amount of 68.98 percent. In this study on 0900 Cherry, Thyme oil found to be the most effective Applications. It was followed by Green Stim, There were no detected any big differences Phenological and pomological (Except fruit weight) observations. Thus, Thyme oil gave the best result for reducing fruit cracking and Green Stim, Chitosan, $CaCl_2$ gave recommendable results compared to the control in 0900 Ziraat cherry cultivar in excessive rainfall years.

REFERENCES

Abdou, E. S., Nagy, K. S. A. And Elsabee, M. Z., 2008. Extraction And Characterization Of Chitin And Chitosan From Local Sources. Bioresource Technology, 99(5): 1359-1367.

Alani, K., 1980. Cracking Of Soft Fruits: Causes And Protective Measures. Obstbau, 5(7):276-278

Anonim, 2010. Http://Www.Tarimvekoyisleribakanligi.Gov.Tr Kiraz Çeşit Kataloğu

Bautista-Banos, S., Hernandez-Lauzardo, A.N., Velazquez-Del Valle, M.G., Hernandez-Lopez, M., Ait Barka, E., Bosquez-Molina, E., Wilson, C.L. (2006). "Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities." Crop Protection, 25(2), 108-118.

Baydar, H., 2007. Tıbbi, Aromatik Ve Keyf Bitkileri Bilimi Ve Teknolojisi. Süleyman Demirel Üniversitesi Ziraat Fakültesi, S.D.Ü. 51:216 S.

Bilginer, S., Demirsoy, L., & Demirsoy, H. (1999). The effects of vapor gard, GA3 and calcium hydroxide applications on fruit cracking in" Turkoglu" sweet cherry. In 3. Turkish National Horticulture Congress in Turkey, Ankara (Turkey), 14-17 Sep 1999. Ankara University.

Bosquez, M. E., Ronquillo, D., Jesus, E., Bautista, B. S., Verde, C. R., Morales, L. J., 2010. Inhibitory Effect Of Essential Oils Against Colletotrichum Gloeosporioides And Rhizopus Stolonifer İn Stored Papaya Fruit And Their Possible Application İn Coatings. Postharvest Biology And Technology, 57: 132-137.

Brown, G., Wilson, S., Boucher, W., Graham, B., Mcglasson, B., 1995. Effect Of Copper-Calcium Sprays On Fruit Cracking İn Sweet Cherry (Prunus Avium L.). Scientia Horticulturae, 62(1995):75-80.

Camele, I., Altieri, L., De Martino, L., De Feo, V., Mancini, E., Rana, G.L., 2012. In Vitro Control Of Post-Harvest Fruit Rot Fungi By Some Plant Essential Oil Components. International Journal Of Molecular Sciences, 13(2): 2290-2300.

Christensen, J.V., 1972a. Cracking İn Cherries. Iıı. Determination Of Cracking Susceptibility, Acta Agriculturae Scadinavica, 22:128-136.

Christensen, J.V., 1972b. Cracking İn Cherries. 4. Physiological Studies Of The Mechanism Of Cracking, Acta Agriculturae Scadinavica 22:153-162.

Christensen, J.V., 1972c. Cracking İn Cherries. The Influence Of Climatic Conditions On Cracking Susceptibility, Saetryk Af Tıdsskrift For Planteavl, 76:191-195.

Christensen, J.V., 1973. Cracking İn Cherries. 4. Cracking Susceptibility İn Relation To Growth Rhythm Of The Fruit, Acta Agriculturae Scadinavica, 23:52-54.

Conway, W.S., Sams, C.E., and Hickey, K.D. (1994). "Preharvest Calcium Sprays for Control of Postharvest Decay in Apples and Pears." Plant Disease, 78(10), 1053-1056.

Davenport, D. C., Uriu, K., And Hagan, R. M., 1972. Antitranspirant Film. Curtailing İntake Of External Water By Cherry Fruit To Reduce Cracking, Hort Scince, 7(5):507-508.

Demirtaş, İ., Sarısu, H.C. Kiraz Yetiştiriciliği. Eğirdir Meyvecilik Araştırma İstasyonu Müdürlüğü Yayınları, Isparta, 2011, Yayın No:11,12 S

El Ghaouth, A., Arul, J., Grenier, J., Asselin, A. (1992). "Antifungal activity of chitosan on postharvest pathogens: Induction of morphological and cytological alterations in Rhizopus stolonifer." Mycological Research, 96(9), 769-779.

Emerce O (2004) Bazı ılıman iklim meyve tür ve çeşitlerinin subtropik koşullardaki performansları. Yüksek Lisans Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Adana

İmrak, B., (2019). The Research on Solution of Multiple Pistil Formation Problems of Stone Fruits. Imternational Science and Research Congress (SR Congress), Alanya, Türkiye, 1:37-45

İmrak, B., Kuden, A., And Sarıer, A., 2018. Researches On 0900 Ziraat Cherry Cultivar Prevent From Fruit Cracking. International Journal Of Agricultural And Natural Sciences, 1(2):142–145.

İmrak, B. ve Kuden, A. (Editörler), 2023. Urban Horticulture – Sustainable Gardening in Cities. IntechOpen, Londra, 186s.

Küden, A., 2004. Cherry Growing Under Subtropic Conditions. Acta Horticulturae, 662:171–175.

Moing, A., Renaud, C., Christmann, H., Fouilhaux, L., Tauzin, Y., Zanetto, A., ... & Claverie, J. (2004). Is there a relation between changes in osmolarity of cherry fruit flesh or skin and fruit cracking susceptibility?. Journal of the American Society for Horticultural Science, 129(5), 635-641.

Özbek, S., 1978. Özel Meyvecilik. Ç.Ü.Ziraat Fakültesi Yayınları No. 128. Ders Kitabı, 483s

Peschel, S., & Knoche, M. (2005). Characterization of microcracks in the cuticle of developing sweet cherry fruit. Journal of the American Society for Horticultural Science, 130(4), 487-495.

Pommier, P., 1989. Sweet Cherry. Experimental Solutions To The Problem Of Cracking, Fos Centre Technique Interprofessionnal Des Fruits Et Legumes 51:11-14.

Ruiz-Aracil, M. C., Valverde, J. M., Lorente-Mento, J. M., Carrión-Antolí, A., Castillo, S., Martínez-Romero, D., & Guillén, F. (2023). Sweet Cherry (Prunus avium L.) Cracking during Development on the Tree and at Harvest: The Impact of Methyl Jasmonate on Four Different Growing Seasons. Agriculture, 13(6), 1244.

Sandford, P. A., 1989. Chitosan: Commercial Uses And Potential Applications, Chitin And Chitosan. Elsevier Applied Science Pub., New York, 51-69s

Saure, M.C. (2005). "Calcium translocation to fleshy fruit: its mechanism and endogenous control." Scientia Horticulturae, 105(1), 65-89.

Sekse, L., 1995. Fruit Cracking İn Sweet Cherries (Prunus Avium L.). Some Physiological Aspects-A Mini Review, Scientia Horticulturae, 63:135-141.

Sivakumar, D. and Bautista-Banos, S. (2014). "A review on the use of essential oils for postharvest decay control and maintenance of fruit quality during storage." Crop Protection, 64, 27-37. DOI: 10.1016/j.cropro.2014.05.012.

TUİK, 2024. Türkiye İstatistik Kurumu Başkanlığı (Presidency of Turkish Statistical Institute). "Tüik Göstergeler". https://www.turkiye.gov.tr/tuik-gostergeler, Accessed: 14.07.2024

Wani, A. A., Singh, P., Gul, K., Wani, M. H., Ve Langowski, H., 2014. Sweet Cherry (Prunus Avium) Critical Factors Affecting The Composition And Shelf Life. Food Packaging And Shelf Life, 1 (1):86-99.

Webster, A.D., Looney, N.E., Ve Kupferman, E.M., 1996. Harvest And Handling Sweet Cherries For The Fresh Market. Cherries: Crop Physiology, Production And Uses, Wallingford, UK, Cab International 411-414.

Westwood, S., 1987. The optimum growing period in the nursery for six important tree species in lowland Nepal. Banko Janakari, 1987, Vol. 1, No. 1, 5-12.

A REVIEW ON PROGRAMMED CELL DEATH IN COTTON

Volkan Mehmet ÇINAR[1], Aydın ÜNAY[2]*

[1] *Postdoctoral Researcher, Faculty of Agriculture, Department of Field Crops, Aydın Adnan Menderes University, Aydın, Türkiye.*
[2] *Faculty of Agriculture, Department of Field Crops, Aydın Adnan Menderes University, Aydın, Türkiye.*
**Corresponding author e-mail: vmcinar@gmail.com*

ABSTRACT

Programmed cell death (PCD) is DNA disruption in which the cell death program is rendered irreversible and facilitates the fragmentation of the nucleus. PCD is actively involved in leaf senescence, self-incompatibility and the formation of tracheary elements in many plants. PCD appears to be mainly involved in cotton fiber development and secondary wall formation. PCD, which occurs under the coordination of Ca^{2+}, hydrogen peroxide, and brassinosteroids, plays a role in the main process leading to fiber maturation following the secondary cell wall. Both developmental-regulated PCD and environmental-induced PCD are affected by several plant hormones. In this review, we have written about gland formation in cotton, its role in anther fertility under high-temperature stress and the role of PCD in the regulation of synergid death. A better understanding of the PCD network will enable us to develop future breeding strategies to improve cotton yield and quality and to increase its tolerance to biotic and abiotic stresses.

Keywords: Cotton, DNA disruption, programmed cell death, reactive oxygen species, stress.

INTRODUCTION

Programmed cell death (PCD) can occur as part of normal growth and development through specific organ-forming and morphological adaptation responses or when cell suicide pathways are activated under the influence of certain biotic and abiotic external factors. PCD can occur in events such as anther maturation during sex determination, pollen tube growth, fertilization, embryo suspension, growth, seed germination, aerenchyma formation, tracheary element and sclereid differentiation, sieve element differentiation, and leaf and flower petal senescence. Apoptosis in animals appears to occur in mycotoxin-treated plant protoplasts and during plant pathogen-induced cell death. It is also argued that apoptosis is not a universal pathway to plant death, and instead, many plant cell suicide programs depend on autophagic and autolytic mechanisms for cell death. Differentiation of xylem conduction bundles, aerenchyma formation, abscission, and senescence are the most important examples of PCDs in the vegetative period. In addition, death of synergids, degeneration of haploid megaspores, and anther dehiscence incompatible pollen tube are also involved in PCD in the generative period.

Programmed cell death (PCD) in plants is a process of cell death regulated by genetics (Buckner et al., 2000; Brodersen et al., 2002) and environmental factors during a specific developmental process (Locato and De Gara, 2018). Locato and De Gara, 2018). PCD frequently occurs during leaf senescence and is a defense mechanism in all field crops, grain formation in cereals, fiber development in cotton, the process by which the nodule provides nitrogen to the plant in leguminous crops such as soybean and aerenchyma formation in rice.

Many researchers have emphasized that multiple signals and phytohormones are involved in the PCD process (Xie et al., 2014; Cortleven et al., 2019; Li et al., 2023). Abiotic stress factors have been reported to trigger PCD (Nath and Lu, 2015; Yanık et al., 2020).

Programmed Cell Death in Cotton Fiber

The development of fibers (Figure 1), which are differentiated seed trichomes in cotton, is completed in 4 developmental stages: initiation, elongation, secondary wall deposition and maturation (Huang et al., 2021; Wen et al., 2023) under the coordination of many factors including reactive oxygen species, brassinosteroids, callose and calcium and arabinogalactans. Elongation of fiber cells, which begins with flowering, continues for about 21-26 days. Secondary cell wall synthesis begins about 16 days after anthesis (DPA), peaks at about day 24 and ends between 32 and 40 (Liu et al., 2024). A drying and ripening period of 45 to 60 DPA occurs (Ji et al., 2003; Kim and Triplett, 2001). Fibers are suggested to die by programmed cell death (PCD) sometime after 40 DPA (Jareczek et al., 2023).

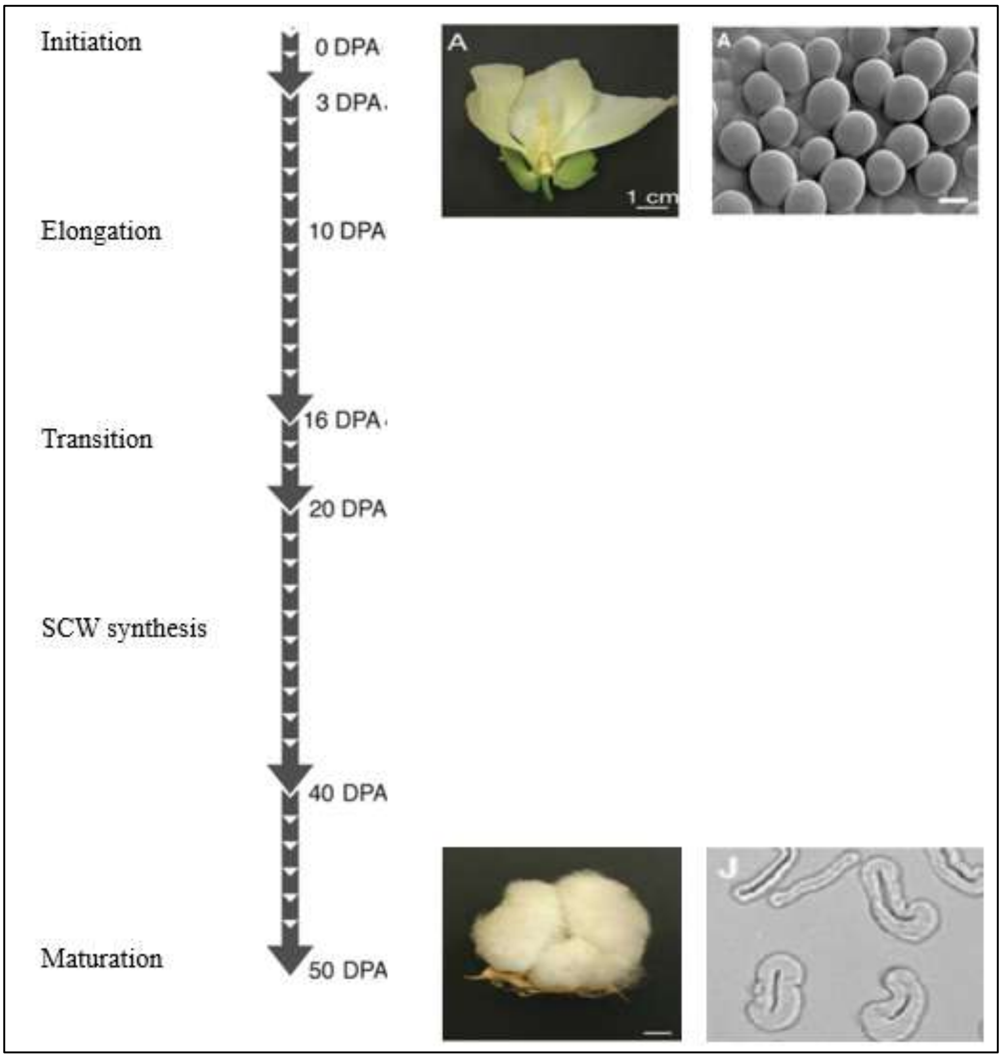


Figure 1. Cotton fiber development (modified from Haigler et al., 2012).

Similarities have been expressed between cotton fiber maturation and tracheary elements, the cells that form xylem vessels characterized by secondary cell wall formation, cytoskeletal reorganization, cellulose accumulation and programmed cell death. Cellular features of programmed cell death mechanisms include nuclear degradation, DNA and cytoplasmic degradation, and caspase-like activity (Love et al., 2008). The central vacuole, which contains most of the intercellular space in cotton fibers, disappears as the cytoplasm dries and is replaced by the lumen as the fiber cells dehydrate between 45 and 60 DPA (Kim and Triplett, 2001).

The Effect of Reactive Oxygen Species

It is hydroxyl radical (OH), a ROS, which plays a role in the remodelling of xyloglucan involved in microfibril attachment, leading to cell elongation (Cosgrove, 1999; Tokumoto et al., 2002; Demidchik et al., 2003). H^+-ATPase activation is involved in the induction of hydrogen peroxide production (Malerba et al., 2004) and acidification of the apoplast, leading to cell wall loosening and, thus, elongation (Rober-Kleber et al., 2003). Hydrogen peroxide (H2O2), notable for its ability to cause cell damage and cell death, has been detected in cotton fiber cells undergoing secondary cell wall synthesis (Potikha et al., 1999).

It was found that UmSrt1 expression in cotton increases the sugar content in the fibers with ROS, and accordingly, early secondary wall synthesis leads to short and thick fibers. It was emphasized that the high quality and long fibers of *G. barbadense* are due to its strong antioxidant capacity (Tuttle et al., 2015; Ding et al., 2021).

Sphingolipids and PCD Association

Sphingolipids, a class of lipid molecules commonly found in eukaryotes and composed of sphingosine, fatty acids and polar head groups, are involved in critical physiological processes, including programmed cell death processes in plants and fiber elongation in cotton (Liang et al., 2003; Wang et al., 2024). It was found that overexpression of GhLCBK1, decreased Sph levels, and increased S1P levels delayed PCD progression in fibers. This delay leads to an increase in fiber length and a decrease in micronaire value. In contrast, suppression of GhLCBK1 expression led to increased Sph content and decreased S1P content. Thus, advancing the PCD process in fibers shortened the elongation time and prolonged the secondary wall synthesis time, which resulted in a decrease in fiber length and an increase in micronaire value (Zhang et al., 2024).

Gland Formation

Pigment gland formation in cotton occurs through a purely lysogenic process (Benedict et al., 2004; Xu et al., 2004). PCD plays a critical role in the lysigenous development of pigment glands in cotton leaves by activating the ROS signalling pathway of H2O2 (Liu et al., 2010; Wang et al., 2016). One of cotton's most important breeding objectives is to obtain gossypol-free seeds. This highly desirable trait increases the value of commercial cotton varieties for both oil and protein utilization from cotton seeds. To achieve this, biotechnological manipulation of CGP1 increases the defense against biotic factors in shoot tissues. It is concluded that the cloning and characterization of CGP1 will be useful in studying the metabolites retained by the gland and their functions in cotton (Gao et al., 2020).

Heat Stress

Anther sterility is caused by altered carbohydrate metabolism or impaired tapetal PCD (Zhang et al., 2010). The Gossypium hirsutum casein kinase I (GhCKI) gene, which encodes a homolog of casein kinase I (CKI), is involved in the inactivation of glycogen synthase and regulation of apoptosis; GhCKI has been indicated as a target gene to improve anther fertility under HT conditions (Min et al., 2013). Two models have been proposed to explain how the stylar S-RNase gains specific access to incompatible pollen RNA. According to both models, the result is a non-autonomous inhibition of protein synthesis, leading to the observed arrest of cell growth and, ultimately, death. Although RNase activity is one of several hydrolytic markers for autolysis during tracheary element differentiation and leaf and petal senescence, stylar S-RNase appears to be the only lytic agent required for a process that could be initiated by the action of a single cell death promoter ± as yet unidentified pollen S-locus product.

Synergid Degeneration

Synergid death has been extensively characterized in cotton. PCD occurs in the embryo sac during fertilisation, and synergid degeneration occurs after pollen tube penetration (Beers, 1997). Significantly in cotton, GA and IAA promote synergid degeneration, with an increase in cytoplasmic density and vacuolar collapse representing early events in synergid death (Jensen et al., 1977).

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In conclusion, PCD plays a crucial role in various developmental processes and responses to environmental stress in plants, including anther maturation, pollen tube growth, seed germination, and fiber development in cotton. The mechanisms underlying PCD in plants often involve complex interactions between genetic and environmental factors, with pathways ranging from autophagy to caspase-like activity. ROS and sphingolipids are particularly significant in regulating these processes, as seen in the differentiation of tracheary elements and the maturation of cotton fibers. Future research should continue to explore the molecular and physiological aspects of PCD, focusing on how specific genes and signalling molecules contribute to cell death pathways. Additionally, understanding the impact of external factors like heat stress and the potential for biotechnological manipulation, such as gland formation in cotton, could lead to advances in crop improvement and stress resistance.

AUTHOR CONTRIBUTIONS

The authors contributed equally to this paper and have read and agreed to the published version of the paper.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

Beers, E. P. 1997. Programmed Cell Death During Plant Growth and Development. Cell Death & Differentiation, 4(8): 649-661. https://doi.org/10.1038/sj.cdd.4400297

Benedict, C.R., G.S. Martin, J. Liu, L. Puckhaber, C.W. Magill. 2004. Terpenoid Aldehyde Formation and Lysigenous Gland Storage Sites in Cotton: Variant with Mature Glands but Suppressed Levels of Terpenoid Aldehydes. Phytochemistry, 65(10): 1351-1359. https://doi.org/10.1016/j.phytochem.2004.03.032

Brodersen, P., M. Petersen, H.M. Pike, B. Olszak, S. Skov, N. Odum, L.B. Jorgensen, R.E. Brown, J. Mundy. 2002. Knockout of Arabidopsis Accelerated-Cell-Death11 Encoding a Sphingosine Transfer Protein Causes Activation of Programmed Cell Death and Defense. Genes & Development, 16(4): 490-502. https://doi.org/10.1101/gad.218202

Buckner, B., G.S. Johal, D. Janick-Buckner. 2000. Cell Death in Maize. Physiologia Plantarum, 108(3): 231-239. https://doi.org/10.1034/j.1399-3054.2000.108003231.x

Cortleven, A., J.E. Leuendorf, M. Frank, D. Pezzetta, S. Bolt, T. Schmülling. 2019. Cytokinin Action in Response to Abiotic and Biotic Stresses in Plants. Plant, Cell & Environment, 42(3): 998–1018. https://doi.org/10.1111/pce.13494

Cosgrove, D.J. 1999. Enzymes and Other Agents that Enhance Cell Wall Extensibility. Annual Review of Plant Biology, 50(1): 391-417. https://doi.org/10.1146/annurev.arplant.50.1.391

Demidchik, V., S.N. Shabala, K.B. Coutts, M.A. Tester, J.M. Davies. 2003. Free Oxygen Radicals Regulate Plasma Membrane $Ca2^{+}$- and K^{+}-Permeable Channels in Plant Root Cells. Journal of Cell Science, 116(1): 81-88. https://doi.org/10.1242/jcs.00201

Ding, X., X. Li, L. Wang, J. Zeng, L. Huang, L. Xiong, S. Song, J. Zhao, L. Hou, F. Wang, Y. Pei. 2021. Sucrose Enhanced Reactive Oxygen Species Generation Promotes Cotton Fibre Initiation and Secondary Cell Wall Deposition. Plant Biotechnology Journal, 19(6): 1092. https://doi.org/10.1111/pbi.13594

Gao, W., F. C. Xu, L. Long, Y. Li, J. L. Zhang, L. Chong, J.R. Botella, C. P. Song. 2020. The Gland Localized CGP1 Controls Gland Pigmentation and Gossypol Accumulation in Cotton. Plant Biotechnology Journal, 18(7): 1573-1584. https://doi.org/10.1111/pbi.13323

Haigler, C. H., L. Betancur, M. R. Stiff, J. R. Tuttle. 2012. Cotton Fiber: A Powerful Single-Cell Model for Cell Wall and Cellulose Research. Frontiers in Plant Science, 3: 104. https://doi.org/10.3389/fpls.2012.00104

Huang, G., J. Q. Huang, X. Y. Chen, Y. X. Zhu. 2021. Recent Advances and Future Perspectives in Cotton Research. Annual Review of Plant Biology, 72(1): 437-462. https://doi.org/10.1146/annurev-arplant-080720-113241

Jareczek, J.J., C.E. Grover, J.F. Wendel. 2023. Cotton Fiber as a Model for Understanding Shifts in Cell Development Under Domestication. Frontiers in Plant Science, 14: 1146802. https://doi.org/10.3389/fpls.2023.1146802

Jensen, W.A., P. Schulz, M.E. Ashton. 1977. An Ultrastructural Study of Early Endosperm Development and Synergid Changes in Unfertilized Cotton Ovules. Planta, 133: 179–189. https://doi.org/10.1007/BF00391917

Ji, S.J., Y.C. Lu, J.X. Feng, G. Wei, J. Li, Y.H. Shi, Q. Fu, D. Liu, J.C. Luo, Y.X. Zhu. 2003. Isolation and Analyses of Genes Preferentially Expressed during Early Cotton Fiber Development by Subtractive PCR and cDNA Array. Nucleic Acids Research, 31(10): 2534-2543. https://doi.org/10.1093/nar/gkg358

Kim, H.J., B.A. Triplett. 2001. Cotton Fiber Growth in Planta and in Vitro. Models for Plant Cell Elongation and Cell Wall Biogenesis. Plant Physiology, 127(4): 1361-1366. https://doi.org/10.1104/pp.010724

Li, C., X. Zhang, W. Guo, K. Fu, C. Li, C. Li. 2023. Impact of Ethylene and Abscisic Acid on Programmed Cell Death in Endosperm and Grain Filling of Wheat (*Triticum aestivum* L.). Journal of Plant Growth Regulation, 42(7): 4275-4289. https://doi.org/10.1007/s00344-022-10891-z

Liang, H., N. Yao, J. T. Song, S. Luo, H. Lu, J. T. Greenberg. 2003. Ceramides Modulate Programmed Cell Death in Plants. Genes & Development, 17(21): 2636-2641. https://doi.org/10.1101/gad.1140503

Liu, L., C. E. Grover, X. Kong, J. Jareczek, X. Wang, A. Si, ... Z. Chen. 2024. Expression Profile Analysis of Cotton Fiber Secondary Cell Wall Thickening Stage. PeerJ, 12: e17682. https://doi.org/10.7717/peerj.17682

Liu, W. Z., Y. F. Zhou, X. Wang, Z. J. Jiao. 2010. Programmed Cell Death During Pigment Gland Formation in *Gossypium hirsutum* Leaves. Plant Biology, 12(6): 895-902. https://doi.org/10.1111/j.1438-8677.2009.00291.x

Locato, V., De Gara, L. 2018. Programmed Cell Death in Plants: An Overview. In: L. De Gara, V. Locato (Ed) Plant Programmed Cell Death. Methods in Molecular Biology. vol 1743. 188 pages. Humana Press, New York. 1–8. https://doi.org/10.1007/978-1-4939-7668-3_1

Love, A. J., J. J. Milner, A. Sadanandom. 2008. Timing is Everything: Regulatory Overlap in Plant Cell Death. Trends in Plant Science, 13(11): 589-595. https://doi.org/10.1016/j.tplants.2008.08.006

Malerba, M., P. Crosti, R. Cerana, R. Bianchetti. 2004. Fusicoccin Affects Cytochrome c Leakage and Cytosolic 14-3-3 Accumulation Independent of H^+-ATPase Activation. Physiologia Plantarum, 120(3): 386-394. https://doi.org/10.1111/j.0031-9317.2004.00261.x

Min, L., L. Zhu, L. Tu, F. Deng, D. Yuan, X. Zhang. 2013. Cotton Gh CKI Disrupts Normal Male Reproduction by Delaying Tapetum Programmed Cell Death via Inactivating Starch Synthase. The Plant Journal, 75(5): 823-835. https://doi.org/10.1111/tpj.12245

Nath, K., Y. Lu. 2015. A Paradigm of Reactive Oxygen Species and Programmed Cell Death in Plants. Journal of Cell Science and Therapy, 6(2): 1-2. http://dx.doi.org/10.4172/2157-7013.1000202

Potikha, T. S., C. C. Collins, D. I. Johnson, D. P. Delmer, A. Levine. 1999. The Involvement of Hydrogen Peroxide in the Differentiation of Secondary Walls in Cotton Fibers. Plant Physiology, 119(3): 849-858. https://doi.org/10.1104/pp.119.3.849

Rober-Kleber, N., T. P. Albrechtova, S. Fleig, N. Huck, W. Michalke, E. Wagner, V. Speth, G. Neuhaus, C. Fischer-Iglesias. 2003. Plasma Membrane H^+-ATPase is Involved in Auxin-Mediated Cell Elongation During Wheat Embryo Development. Plant Physiology, 131(3): 1302-1312. https://doi.org/10.1104/pp.013466

Tokumoto, H., K. Wakabayashi, S. Kamisaka, T. Hoson. 2002. Changes in the Sugar Composition and Molecular Mass Distribution of Matrix Polysaccharides During Cotton Fiber Development. Plant and Cell Physiology, 43(4): 411-418. https://doi.org/10.1093/pcp/pcf048

Tuttle, J.R., G. Nah, M.V. Duke, D.C. Alexander, X. Guan, Q. Song, Z.J. Chen, B.E. Scheffler, C.H. Haigler. 2015. Metabolomic and Transcriptomic Insights into How Cotton Fiber Transitions to Secondary Wall Synthesis, Represses Lignification, and Prolongs Elongation. BMC Genomics, 16: 1-28. https://doi.org/10.1186/s12864-015-1708-9

Wang, L., C. Jin, W. Zhang, X. Mei, H. Yu, M. Wu, W. Pei, J. Ma, B. Zhang, M. Luo, J. Yu. 2024. Sphingosine Promotes Fiber Early Elongation in Upland Cotton. Plants, 13(14): 1993. https://doi.org/10.3390/plants13141993

Wang, L.L., S.S. Zheng, P.P. Tong, C. Yan, W.Z. Liu. 2016. Cytochemical Localization of H2O2 in Pigment Glands of Cotton (*Gossypium hirsutum* L.). Journal of Integrative Agriculture, 15(7): 1490-1498. https://doi.org/10.1016/S2095-3119(15)61210-2

Wen, X., Z. Chen, Z. Yang, M. Wang, S. Jin, G. Wang, L. Zhang, L. Wang, J. Li, S. Saeed, S. He. 2023. A Comprehensive Overview of Cotton Genomics, Biotechnology and Molecular Biological Studies. Science China Life Sciences, 66(10): 2214-2256. https://doi.org/10.1007/s11427-022-2278-0

Xie, Y., C. Zhang, D. Lai, Y. Sun, M.K. Samma, J. Zhang, W. Shen. 2014. Hydrogen Sulfide Delays GA-Triggered Programmed Cell Death in Wheat Aleurone Layers by the Modulation of Glutathione Homeostasis and Heme Oxygenase-1 Expression. Journal of Plant Physiology, 171(2): 53-62. https://doi.org/10.1016/j.jplph.2013.09.018

Xu, Y.H., J.W. Wang, S. Wang, J.Y. Wang, X.Y. Chen. 2004. Characterization of GaWRKY1, a Cotton Transcription Factor that Regulates the Sesquiterpene Synthase Gene (+)-δ-Cadinene Synthase-A. Plant Physiology, 135(1): 507-515. https://doi.org/10.1104/pp.104.038612

Yanık, F., A. Çetinbaş-Genç, F. Vardar. 2020. Abiotic Stress–Induced Programmed Cell Death in Plants. D.K. Tripathi, V.P. Singh, D.K. Chauhan, S. Sharma, S.M. Prasad, N.K. Dubey, N. Ramawat (Ed) Plant Life Under Changing Environment: Responses and Management. 994 pages. Academic Press, Massachusetts. 1-24. https://doi.org/10.1016/B978-0-12-818204-8.00015-1

Zhang, H., W. Liang, X. Yang, X. Luo, N. Jiang, H. Ma, D. Zhang. 2010. Carbon Starved Anther Encodes a MYB Domain Protein that Regulates Sugar Partitioning Required for Rice Pollen Development. Plant Cell, 22(3): 672–689. https://doi.org/10.1105/tpc.109.073668

Zhang, J., Q. Meng, Q. Wang, H. Zhang, H. Tian, T. Wang, F. Xu, M. Luo. 2024. Cotton Sphingosine Kinase GhLCBK1 Participates in Fiber Cell Elongation by Affecting Sphingosine-1-Phosphate and Auxin Synthesis. International Journal of Biological Macromolecules, 267: 131323. https://doi.org/10.1016/j.ijbiomac.2024.131323

FIELD RESISTANCE OF BARLEY VARIETIES TO LEAF RUST

Vanya IVANOVA, Yordanka BACLAROVA

Agricultural Academy, Dobrudzha Agricultural Institute – General Toshevo, 9521, Bulgaria

**Corresponding author e-mail: vkiryakova@yahoo.com*

ABSTRACT

Leaf rust is an economically important disease on barley in Bulgaria. Since cultivated barley *Hordeum vulgare* is considered nonhost or an intermediate type of host of leaf rust on wheat *P. triticina* and is to some extent attacked by this heterologous fungus as well, our studies were carried out in an infection field of Dobrudzha Agricultural Institute – General Toshevo, Bulgaria, where wheat, triticale and barley materials were planted. The study was conducted in three consecutive harvest years, and the infection load included the natural population of *P. hordei* and the artificial and natural population of *P. triticina.* Thirty-four barley varieties of Bulgarian and foreign breeding were subjected to screening for resistance to leaf rust. The final disease attack, the average coefficient of infection and the resistance rate of these varieties were measured. The varieties were divided into four groups depending on the manifested resistance to the pathogens.
The four groups of varieties demonstrated different variations within the resistant type. Therefore, all investigated Bulgarian barley varieties, including also three varieties of foreign breeding, can be involved in breeding programs for developing of improved cultivars, which carry resistance to leaf rust.

Key words: Barley, cultivars, field resistance, *P. hordei*

INTRODUCTION

Barley is a main field crop in Bulgaria. Besides being a food-supply crop, it is also demanded on the market as a raw material for the brewing industry, as valuable animal food and as raw material for production of cellulose (Stefanov et al., 1986). The export of barley grain stimulates the interest of the producers and these advantages define it as a promising and strategic crop for the country under the conditions of modern agriculture. On a world scale, the crop ranks fourth by production, following wheat, maize and rice (Shulte et al., 2009). Traditionally, the EU is a leading exporter of malting barley to the world market. Bulgaria is able to meet the demands of its internal market almost entirely by own production. During the last 10-15 years, the areas sown with barley in Bulgaria varied within 2.5 – 4.0 million ha, according to data provided by the Ministry of Agriculture and Foods.
Barley production is often limited by various factors, some of which are the annual or sporadic occurrence of diseases. Although leaf rust caused by *P. hordei* is of sporadic occurrence, it may also be common and widespread disease on barley. The crop is often attacked by *P. triticina*, which is a biotrophic pathogen capable of rapid change, quickly becoming virulent to resistant hosts. Within the plant species, which are nonhosts to a given pathogen, sometimes there are genotypes moderately susceptible to a heterologous pathogen (Dracatos et al., 2016). Cultivated barley *Hordeum vulgare* is considered nonhost or an intermediate type of host for leaf rust on wheat *P. triticina*.

Studies have shown that it is sometimes attacked by *P. triticina* and some other heterologous fungi such as *P. hordei-murini*, *P. hordei-secalini*, etc. (Atienza et al., 2004; Jafari et al., 2006). The investigations of Neu et al. (2003) proved that there are plant species, which are resistant to most of the pathogens found in their environment. They believe that the resistance, which is not specific for the pathogen, is maintained through a combination of passive and active defense mechanisms; they called it resistance without a host.

In the past decades, an increase of leaf rust attacks has been observed in the regions with moderate growing of barley due to the more intensive agronomy practices. Although the disease does not lead to full loss of harvest, data has been reported indicating that yield decrease can be as high as 60% and more in the susceptible varieties under conditions of epidemics (Clifford, 1985; Cotterill et al., 1995; Griffey et al., 1994; Park et al., 2015). The development of varieties possessing a complex of valuable economic properties and characteristics, which are able to realize their production potential under the varied meteorological conditions of Bulgaria, is a main goal of cereals breeding, and of barley breeding, in particular.

Although in the recent years an increasing number of varieties of European breeding which set higher requirements of adaptability of the productivity and grain quality-related characters and properties are being introduced. As a result from the thorough and purposeful breeding work in Bulgaria, a number of varieties were developed, which are highly adaptable to the conditions of our country and largely meet the production standards, and which are carriers of such valuable properties as disease resistance (Baklarova Y., G., Mihova, 2020; Dimitrova-Doneva et al., 2012; Dyulgerova and Valchev, 2012; Gocheva, Valchev, 2014; Mihova et al., 2011; Valchev and Valcheva, 2010; Valchev and Gocheva, 2012; Valcheva and Valchev, 2009, 2010; Valchev and Dyulgerova, 2011; Valcheva et al., 2012).

The short lifespan of the resistance determined by race-specific genes is the reason why breeders keep on searching for other types of resistance, such as partial resistance, for instance, which is more durable and race non-specific (Parlevliet and van Ommeren, 1975).

The aim of this study was to test under field conditions some of the barley varieties developed in Bulgaria, as well as some foreign cultivars for resistance to leaf rust, so that the data obtained could be useful to the breeding for improved varieties with higher resistance to this pathogen.

MATERIAL AND METHOD

The investigation was carried out during 2019-2021 in an infection field at Dobrudzha Agricultural Institute – General Toshevo. There were natural populations of *P. hordei* and natural and artificial population of *P. triticina* present. Thirty-four barley varieties were subjected to testing for leaf rust; they are presented in Table 1.

Table 1. Barley varieties used in the study

Origin	Cultivar/ Variety
Institute of Agriculture - Karnobat	Izgrev, Veslets, Hemus, Obzor, Emon, Asparuh, Deviniya, Zagorets, Imeon, Kuber, Lardia, Odesey, Orphey, Perun, Sayra, Aheloy 2, Bori, Bozhin, Panagon
Dobrudzha Agricultural Institute-G. Toshevo	Kaskadyor 3, Radul, Pagane, Tangra, Ahat, Yaspis, Onyx, Fanagoria
Agrarian University - Plovdiv	Kristy, Kramy
Institute of Plant and Genetic Resources - Sadovo	Potok
Agrodimex Ltd	Flaviy
Foreign breeding	Vicky, Vanessa, Malvinta

The varieties were sown manually, in 1.5 m rows with 0.25 cm interspacing. Cultivar *Michigan amber* was used as a multiplier and propagator of the rust and was planted across every 10 cultivars or breeding lines, as well as along the plots. The artificial inoculation with the pathogen was performed according to a methodology adopted at the Plant Pathology laboratory of Dobrudzha Agricultural Institute for working with rusts (Ivanova, 2012).

The type of infection and the attacking rate were read according to Cobb's scale, modified by Peterson (1948), at milk maturity stage. Disease severity was assessed as a percentage of leaf area, covered by the rust uredinia. The average coefficient of infection (ACI) or the so called corrected relative attacking rate was calculated by introducing coefficients for the respective infection types (R-0.2; MR-0.4; M-0.6; MS-0.8; S-1).

Depending on the ACI values, the studied varieties were divided into several groups: Immune (ACI=0); very resistant VR (ACI = 0–5.99); resistant R (ACI = 6–25.99); moderately resistant MR (ACI=26–45.99); moderately susceptible MS (ACI = 46– 65.99); susceptible S (ACI = 66–100). The attacking rate of the standard susceptible cultivar *Michigan amber* was 60 – 100% over the years of investigation.

RESULTS AND DISCUSSION

The disease severity, ACI and the rating of the barley varieties tested under field conditions are given in Table 2.

Table 2. Field resistance of barley cultivars under infection field conditions

Cultivar	2019			2020			2021		
	DS	ACI	Rating	DS	ACI	Rating	DS	ACI	Rating
Izgrev	0	0	VR	15/4	16.7	R	5/4	8.3	R
Veslets	5/4	5.0	VR	5/4	6.3	R	5/4	8.3	R
Hemus	5/4	5.0	VR	5/4	6.3	R	0	0	VR
Obzor	0	0	VR	10/4	11.1	R	0	0	VR
Emon	5/4	5.0	VR	25/4	27.8	MR	5/4	8.3	R
Asparuh	5/4	5.0	VR	25/4	27.8	MR	0	0	VR
Ahat	10/4	10.0	R	25/4	27.8	MR	0	0	VR
Vanessa	0	0	VR	5/4	6.3	R	0	0	VR
Vicky	10/4	10.0	R	5/4	6.3	R	0	0	VR
Deviniya	25/4	25.0	R	25/4	27.8	MR	5/4	8.3	R
Zagorets	5/4	5.0	VR	15/4	16.7	R	5/4	8.3	R
Imeon	5/4	5.0	VR	40/4	44.4	MR	10/4	16.6	R
Kramy	25/4	25.0	R	30/4	33.3	MR	5/4	8.3	R
Kristy	5/4	5.0	VR	60/4	66.7	S	10/4	16.6	R
Kuber	5/4	5.0	VR	30/4	33.3	MR	5/4	8.3	R
Lardea	5/4	5.0	VR	40/4	44.4	MR	0	0	VR
Malvinta	10/4	10.0	R	5/4	6.3	R	10/4	16.6	R
Odisey	5/4	5.0	VR	30/4	33.3	MR	10/4	16.6	R
Orfey	0	0	VR	40/4	44.4	MR	5/4	8.3	R
Perun	0	0	VR	40/4	44.4	MR	15/4	25.0	R
Potok	0	0	VR	60/4	66.7	S	10/4	16.6	R
Sayra	0	0	VR	25/4	27.8	MR	0	0	VR
Flaviy	10/4	10.0	R	40/4	44.4	MR	10/4	16.6	R
Aheloy 2	10/4	10.0	R	10/4	11.1	R	5/4	8.3	R
Bori	10/4	10.0	R	10/4	11.1	R	5/4	8.3	R
Bozhin	5/4	5.0	VR	30/4	33.3	MR	0	0	VR
Panagon	0	0	VR	0	0	VR	10/4	16.6	R
Kaskadyor 3	0	0	VR	25/4	27.8	MR	10/4	16.6	R
Radul	10/4	10.0	R	0	0	VR	5/4	8.3	R
Pagane	25/4	25.0	R	25/4	27.8	MR	5/4	8.3	R
Yaspis	5/4	5.0	VR	40/4	44.4	MR	0	0	VR
Onyx	5/4	5.0	VR	15/4	16.7	R	15/4	25.0	R
Tangra	10/4	10.0	R	15/4	16.7	R	10/4	16.6	R
Fanagoria	0	0	VR	30/4	33.3	MR	5/4	8.3	R
M. amber	100/4	100	VS	90/4	100	VS	60/4	100	VS

Depending on the final attacking rate and the average coefficient of infection, several groups of varieties were formed, which responded in certain ways.

The first group included varieties with varied reaction: from very resistant to resistant (VR – R) – Izgrev, Veslets, Hemus, Obzor, Zagorets, Panagon (breeding of Institute of Agriculture – Karnobat); Radul and Onyx (developed at Dobrudzha Agricultural Institute – General Toshevo), as well as the varieties of foreign breeding Vicky and Vanessa.

Within the second group fell varieties with stable reaction of resistance (R) in all three years of the study: Aheloy 2 and Bori (breeding of Institute of Agriculture – Karnobat); variety Tangra (developed at Dobrudzha Agricultural Institute – General Toshevo) and the foreign cultivar Malvinta.
The third group included varieties, which responded with high to moderate resistance (VR-MR). These were Asparuh, Lardea, Bozhin and Saira – breeding of Institute of Agriculture – Karnobat, as well as cultivar Yaspis – breeding of Dobrudzha Agricultural Institute – General Toshevo). With changeable reaction, though within the resistant type (VR-R-MR), a fourth group was formed during the years of studied, including varieties Imeon, Kuber, Odisey, Orfey, Emon, Perun, Deviniya – breeding of Institute of Agriculture – Karnobat, Kaskadyor 3, Ahat, Pagane and Fanagoria – breeding of Dobrudzha Agricultural Institute – General Toshevo; cultivar Flaviy developed at Agrodimex company and cultivar Krami developed at the University of Agriculture – Plovdiv. Cultivars Deviniya, Pagane and Krami responded with resistance type R-MR (Table 2).
Cultivar Deviniya is a two-rowed barley. Its authors pointed out that the variety was highly resistant to leaf rust under field conditions (Valcheva and Valchev, 2013). Our investigation confirmed that against artificial infection background this cultivar demonstrates resistant to moderately resistant reaction within the 3-year period of investigation. During the previous 3-year period, cultivars Pagane, Krami and Deviniya responded with very resistant to resistant reaction (VR-R) in the infection field (unpublished data). This new investigation revealed that the resistance during the current period of time was R-MR.
Two varieties, Potok, developed by the Institute of Plant and Genetic Resources – Sadovo and Kristy, developed at the Agrarian University – Plovdiv, responded with a susceptible reaction in one of the years, while in the other two years they demonstrated high resistance (Table 2). In the preceding 3-year period, these two cultivars responded as very resistant to resistant (unpublished data), but due to these differences registered in this study and the occurrence of susceptibility, they were not included in any of the groups formed.
It is noteworthy that the high susceptibility of these two cultivars was manifested in the same year, when a part of the varieties also reacted with a higher rate of attack than usual – up to moderate resistance (MR). It is possible that more aggressive pathotypes occurred in the respective year in the natural population of the pathogen; these pathotypes might have overcome the resistance of certain varieties, and the high susceptibility manifested as moderate.

CONCLUSIONS

Under the conditions of Bulgaria, all Bulgarian barley varieties tested for resistance to leaf rust, as well as the foreign cultivars Vicky, Vanessa and Malvinta, demonstrated good resistance to the natural population of *P. hordei* and the natural and artificial population of *P. triticina* in the field. They could all be involved in breeding programs for higher genetic diversity and developing of improved barley varieties, which carry resistance to leaf rust.

REFERENCES

Atienza S. G., H. Jafary and R. E. Niks. 2004. Accumulation of genes for susceptibility to rust fungi for which barley is nearly a nonhost results in two barley lines with extreme **multiple susceptibility, Planta 220:71-**79.

Baklarova Y., G. Mihova. 2020. Economic characterization of a new winter feed barley Fanagoriya. Field Crops Studies, XIII (1), 91-98.

Clifford B. C.1985. Diseases, distribution, epidemiology and control. Burley Leaf Rust, 173-205. https://doi.org/10.1016/b978-0-12-48402-6.50014-6

Cotterill P. J., Park R. F., Rees R. G.1995. Pathogenic Specialization of *Puccinia hordei* Othh. In Autralia,1966-1990. Australian Journal of Agricultural Research 46, 127-134.

Dimitrova-Doneva, M., Valcheva D., Valchev D., Dimova D., Dyulgerova B. 2012. Productive capacity of varieties and perspectives lines of feld barley. Scientific works of the Institute of Agriculture-Karnobat, 1 (1), 71-77.

Dracatos P. M., M. Nansamba, A. Berlin, R. F. Park and R. E. Niks. 2016. Isolate Specificity and Polygenic Inheritance in Barley to the Heterologous Rust Pathogen *Puccinia graminis* f.sp. *avenae*. The American Phytopathological Society, vol.106, № 9, 1029-1037. https://doi.org/10.1094/PHYTO-10-15-0264-R

Gocheva M., Dr. Valchev. 2014. Characterisation of winter malting barley cultivar Kuber. Scientific works of Institute of Agriculture-Karnobat, 3, (1), 71-76 (in Bg).

Griffey C. A., Das M. K., Baldwin R. E. and Waldenmaier C. M. 1994. Yeld losses in winer barley resulting from a new race of *Puccinia hordei* in Nortch America. Plant Disease 78, 256-260.

Ivanova V. 2012. Studies on the resistance of common wheat and other species to the cause agent of brown rust *Puccinia triticina* Erikss. PhD thesis, DAI- General Toshevo, 2012 (in Bg).

Jafary H., L. Szabo and R. E. Niks. 1998. Specificity of prehaustorial resistance to *Puccinia hordei* and to two inappropriate rust fungi in barley. Phytopathology 88:856-861.

Neu C., C. Feuillet, B. Keller. 2003. Cytological and Molecular Analysis of the *Hordeum vulgare - Puccinia triticina* Nonhost Interaction. The American Phytopathological Society Molecular Plant Microbe Interactions, vol. 16, No 7: 626-633. https://doi.org/10.1094/MPMI.2003.16.7.626.

Parlevliet, S. E., A. van Ommeren. 1975. Partial resistance of barley to leaf rust, *Puccinia hordei.* II. Relationship between field trials,microplot test and latent period. Euphytica 24, 203-303.

Park R., P. G. Golegaonkae, L. Derevnina, K. S. Sandhu, H. Karaoglu, H. M. Elmansour, P. M. Draeatos, D. Singh. 2015. Leaf Rust of cultivated barley: pathology and control. Ann. Rev. Phytopathoogy 53:565-89. https://doi.org/10.1146/annurev-phyto-080614-120324

Peterson R. F., A. B. Campbell, A. E. Hannah. 1948. A diagramic scale for estimating rust intensity on leaves and stems of cereals. Can. J. Res. Sect. C., 26: 495-500.

Shulte D., Timothy J. Close, A. Graner, P. Langridge, T. Matsumoto, G. Muchlbauer, K. Sato, A. H. Shulman, R. Waugh, R. P. Wise, N. Stein. 2009. The International Barley Sequencing Consortium – At the Threshold of Efficient Access to the Barey Genome Plant Physiology, Vol. 149, Issue 1, pp. 142-147. https://doi.org/10.1104/pp.108.128967

Stefanov T. G. and B. Gramatikov. 1986. Barley in Bulgaria. Importance, distribution and yields. Zemizdat, Sofia, 1986. – (Bg).

Valcheva D., Valchev Dr., Michova G., Doneva M. & Dyulgerova, B. 2012. Productive capacity of the winter malting barley varieties in terms of the Northeast and Southeast Bulgaria. Field Crop Studies, 8, (2), 209-217.

Valcheva D., Valchev D. 2009. Lardeya – a new Bulgarian winter malting barley variety. Plant breeding science, 5, 475-480.

Valcheva D., Valchev D. 2010. Sayra – a new Bulgarian winter malting barley variety. Plant breeding science, 5, 473-477.

Valcheva D., Valchev D. 2013. Deviniya – a new winter malting barley variety. Scientific works of the Institute of Agriculture-Karnobat, 1 (2), 163-168.

Valchev D., D. Valcheva. 2010. Orfey – the first Bulgarian drought resistant barley variety, Plant breeding science, 2, 187-189 (in Bg).

Valchev D., M. Gocheva. 2012. Biological and economic properties of the new malting barley cultivar Asparuh. Plant breeding science, 1, 13-19 (in Bg).

Valchev D., B. Dyulgerova. 2011. Bori – a new winter forage barley variety. Plant breeding science, 5, 427-430 (in Bg).

Dyulgerova B. Valchev D. 2012. Characterization of winter forage barley Bozhin. Plant breeding science, 2, 49, 62-66 (in Bg).

Mihova G., S. Tsvetkov, T. Petrova, I. Iliev, V. Ivanova, S. Doneva, V. Bachvarova, G. Marinova, A. Krasteva. 2011. Malting barley variety Kaskadyor 3 combining high productivity and quality. Field Crop Studies vol VII-1, 49-61 (in Bg).

HYDROCHEMICAL WATER QUALITY USE IN AGRICULTURAL ACTIVITIES IN THE NORTH-EAST OF ALGERIA: EL EULMA CITY.

Samia BOUNAB[1*], Kaddour KHEMMOUDJ[2]

[1]Research Laboratory of Applied Hydraulics and Environment, Faculty of Technology, University of Bejaia, 06000 Bejaia, Algeria.
[2]Laboratory of Applied Zoology and Animal Ecophysiology, Faculty of Life and Nature Sciences, University of Béjaia, Béjaia 06000, Algeria.
[*]Corresponding author's email: **Samia.bounab@univ-bejaia.dz**

ABSTRACT

The study area is located at 25 km to El Eulma City, in the North - East of Algeria, where there exists a strong demand for water due to the socio-economic development of region. The development of agricultural has led to a notable use of water resources and lead farmers to use groundwater for irrigation of crops, which may affect the physico-chemical quality of groundwater and soil. In 2022, a sampling campaign conducted to assess the quality of groundwater for irrigation purposes. To achieve this objective, twenty groundwater samples were collected and analyzed for physical (pH, EC, TDS) and chemical (Na^+, K^+, Ca^{2+}, Mg^{2+}, HCO_3^-, Cl^-, SO_4^{2-}, NO_3) parameters. The analysis results are processed using hydro-chemical and multivariate statistical methods. These analyses show that the tendency of the cations in the most samples are in the order of $\mathbf{Ca^{++} > Na^+ > Mg^{++} > K^+}$**.** However, tendency of anions is in the order of $\mathbf{HCO_3^- > SO_4^{--} > Cl^-}$**.** Evaluation of the water types using stabler diagram, show that the majority of the samples represents the $\mathbf{HCO_3^-\ Ca^{++}}$ type of water. The suitability of groundwater for irrigation was determined according to a number of parameters such as salinity, sodium adsorption ratio, sodium percentage, residual sodium carbonate, permeability index, Kelly's ratio, potential salinity and magnesium hazard. The study concluded that the water from the study area is good and suitable for irrigation with few exceptions.

Keywords: Groundwater, irrigation water quality, agricultural activities, facies, El Eulma City.

INTRODUCTION

Groundwater accounts for 43% of the world's irrigation water and is better suited for irrigation purposes than surface water, it is clear that the quality of agricultural water has an impact on soil quality and thus on the harvests obtained. To avoid the negative impacts on soil quality and plants, it is important to monitor the water quality for irrigation. In this regard, the current research aimed two objectives: (i) to determine the hydro chemical characteristics of groundwater, (ii) to use a variety of methods for the overall assessment and monitoring of groundwater quality for irrigation.

- **Study area**

The studied area is located in the Eastern of Algeria (Fig.1). The climate of the study area is classified as semi-arid climate, with an annual precipitation ranging from 300 to 420 mm/year, and the average monthly temperature varies between -5 and 40 OC. The variability lithologic in the aquifer may have an impact on water composition because of its effect on rock/water interactions and directions of groundwater movement.

- **Sampling**

A total of twenty groundwater samples (Fig.1), were collected from private wells and analyzed for physical and chemical parameters. These parameters were determined using standard techniques recommended by Rodier (1996). The analysis results are processed using hydro-chemical and multivariate statistical methods.

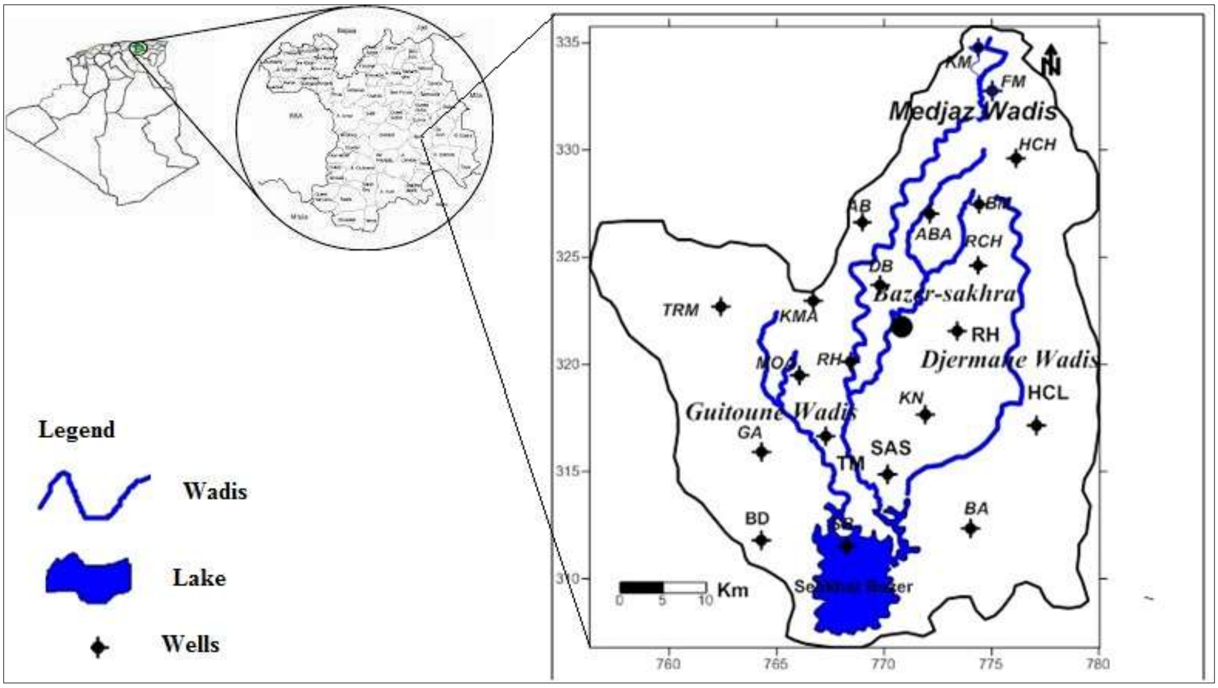


Fig. 1 Location of the studied site and the groundwater sampling location

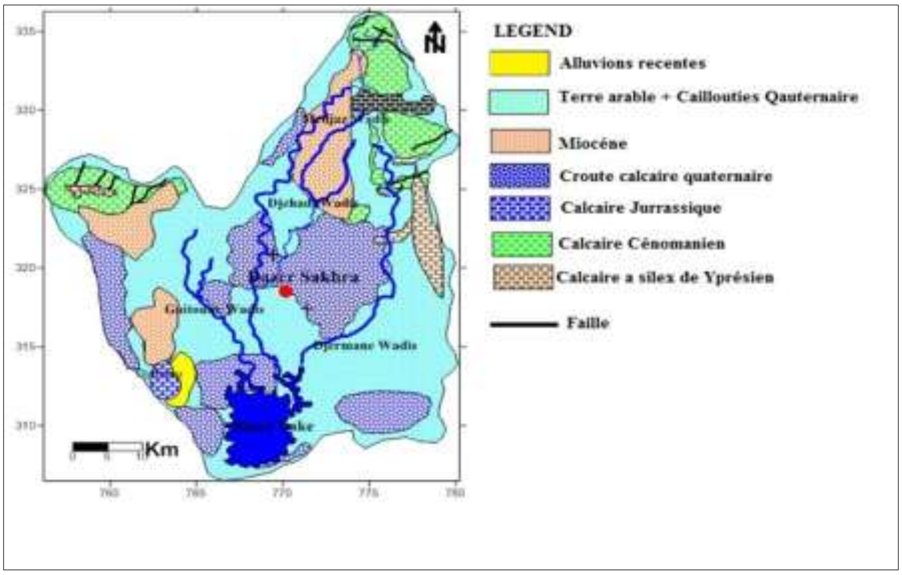


Fig. 2 Geological map of the studied area

- **Hydrochemistry of the groundwater samples**

These results indicate that, the cations tendency in the most of groundwater samples is in the order of
Ca2+>Na+>Mg2+>K+, which represent 70%, The tendency of anions is in the order of HCO3 ->SO4 2->Cl- represent 50% followed by HCO3 >Cl-> SO42--.Stabler (1944) diagram (Fig. 3) shows that, HCO3 - Ca2 type of water was predominant in the majority of samples (80%).

- **Irrigation groundwater quality**

The quality groundwater for irrigation purposes was evaluated and discussed by determining a several parameters such as, %Na+, SAR, RSC, PI, KR, PS, MH. Table1

Table 1**.** Statistics of the several groundwater parameters for irrigation

Variable	Min	Max	Mean
%Na	8.640	63.020	33.970
KR	0.050	1.655	0.539
SAR	0.240	11.980	2.372
MH	11.847	48.342	31.199
PI	26.946	75.387	52.314
PS	1.260	80.625	7.640
RSC	-44.300	-5.011	-9.200

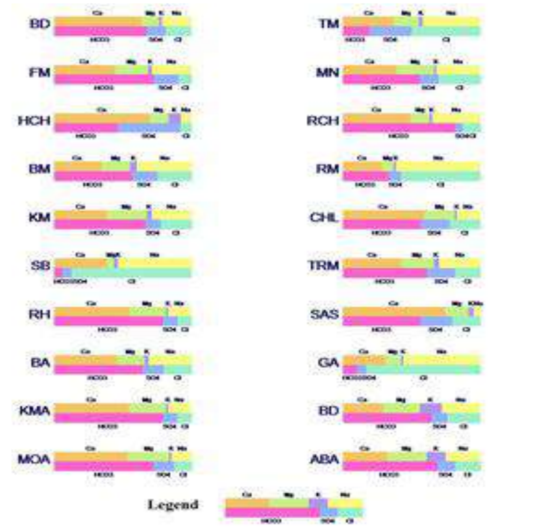


Fig. 3 Stabler diagram illustrating major ionic dominance in the samples

These irrigation parameters were calculated as follows: % Na was calculated using Eq.1 (Wilcox, 1955).

$$\%Na^{+} = (Na^{+} + K^{+}) \times \frac{100}{Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}} \qquad (1)$$

SAR (Richards, 1954). It was calculated using the Eq. 2

$$SAR = Na^{+}/\sqrt{Ca^{2+} + Mg^{2+}}/2 \qquad (2)$$

RSC was computed using Eq. 3 (Ragunath, 1987).

$$RSC = (HCO_3^{-} + CO_3^{-}) - (Ca^{2+} + Mg^{2+}) \qquad (3)$$

MH was calculated using Eq. 4 (Szaboles & Darab, 1964).

$$MH = \frac{Mg^{2+}}{(Ca^{2+} + Mg^{2+})} \times 100 \qquad (4)$$

KR was computed using Eq. 5 (Kelly, 1940, 1963).

$$KR = \frac{Na^{+}}{(Ca^{2+} + Mg^{2+})} \qquad (5)$$

PI was computed using Eq. 6 (Doneen, 1964).

$$PI = Na^{+} + \sqrt{HCO_3^{-}}/(Ca^{2+} + Mg^{2+} + Na^{+}) \times 100 \qquad (6)$$

PS was calculated using Eq. 7 (Doneen, 1964).

$$PS = Cl^{-} + \sqrt{SO_4^{2-}} \qquad (7)$$

In these equations, all ionic concentrations were represented in mill equivalents per liter.

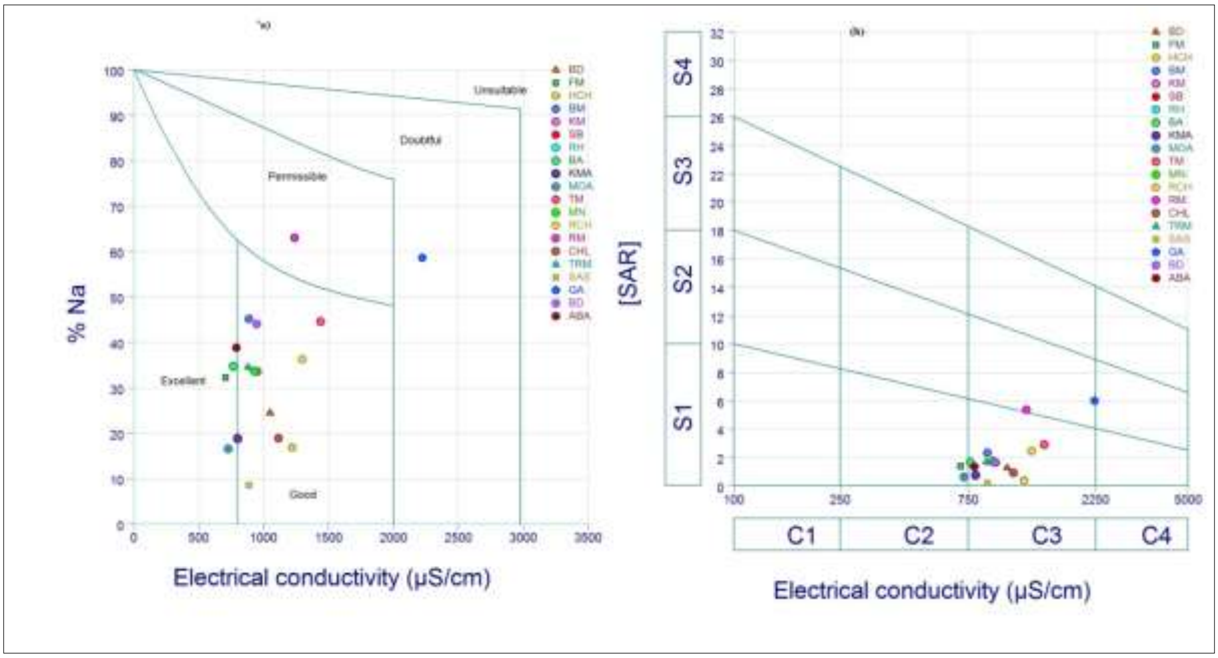


Fig. 4 Wilcox diagram (a) and Richards's diagram (b) of groundwater samples

Discussion

- ✓ The Na % values of samples ranged from 8.64 to 63.02 with mean of 33.97, 95 % of samples fall in "excellent" and "permissible" category (Table 1)
- ✓ *Sodium Adsorption Ratio (SAR)* values vary from 0.24 to 11.98 mg/l with a mean of 2.37 mg/l (Table 1). the majority of samples fall into excellent (95 %) to good (5%) classes similar to Na% classification.

- ✓ The electrical conductivity and SRA values plotted show that the most of the water samples belong into the C2S1class "medium salinity - low sodium" and C3S1 "high salinity - low sodium", According to the figure (Fig 4, b), all of the groundwater samples could be utilized for irrigation on practically every soil.

- ✓ Wilcox diagram, reveals that all groundwater samples fall in "excellent "to "permissible" category except one water sample (Fig.4),
- ✓ *The RSC* values of RSC range from (- 44.30 to - 5.01), these values are less than 1.25 meq/l (Table 1*)*, the entire water samples fall in good water category, "not hazard "so suitable for irrigation use
- ✓ *The MH* values range from 11.85 and 48.34 with a mean of 31.19 (Table 1). These values obtained are less than 50 and show that all groundwater samples are "good and suitable" for irrigation

- ✓ *Kelly's ratio (KR)* values are more than 1 in 85 % of the groundwater samples, the vast majority of samples from the studied area are suitable for irrigation use.
- ✓ *Permeability Index (PI)* values of the samples range from 26.94 to 75.4 with a mean of 52.31. It revealed that, 100 % of water samples fall into "good" water category which indicates that the water is suitable for irrigation purposes

- ✓ *Potential Salinity (PS)* values of groundwater samples range from 1.26 to 80.6 with a mean of 7.64

(Table 1).65% and 15% of the samples were classified as "excellent" to "good", and were "good" to "injurious" category of water quality respectively

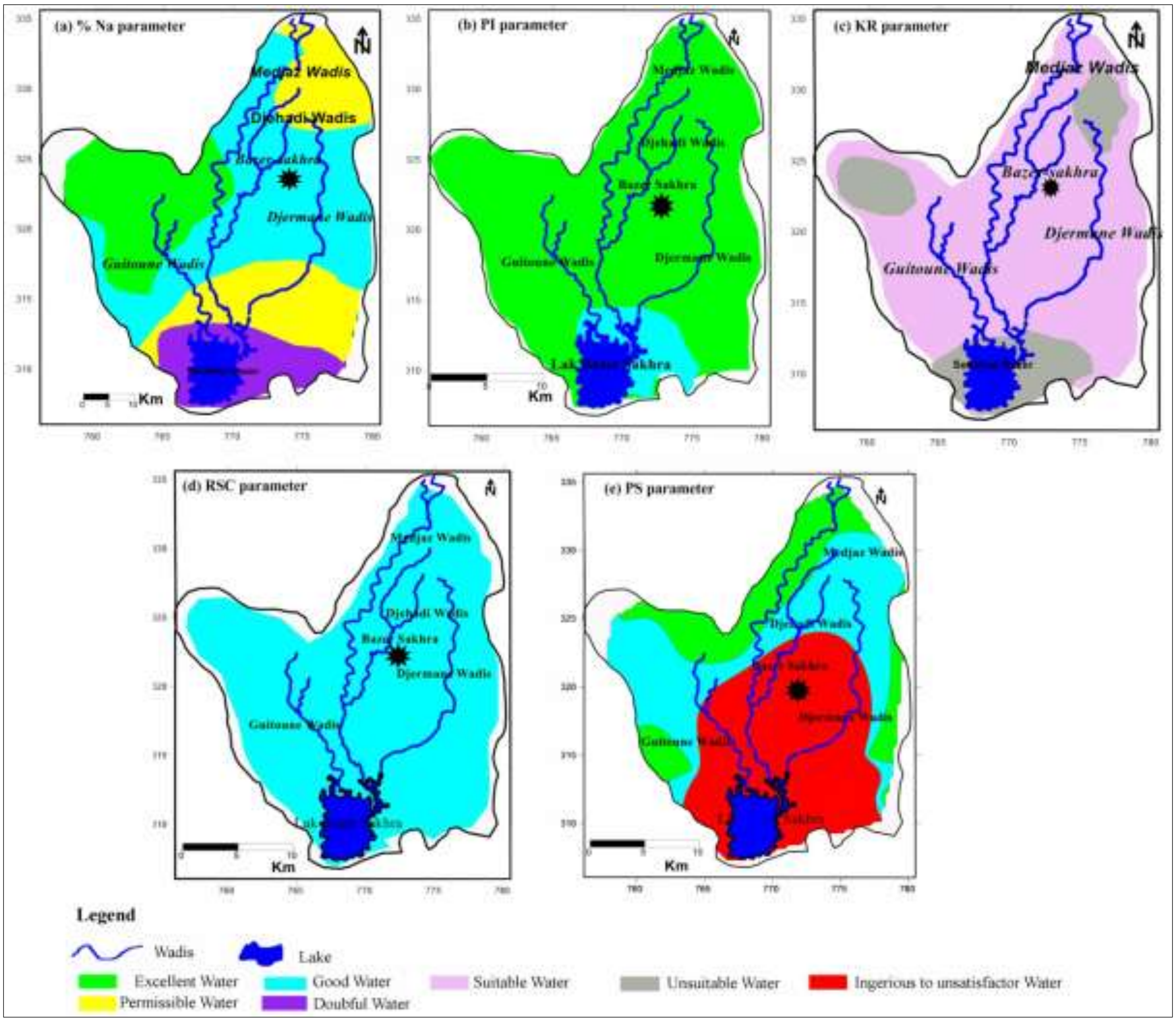


Fig. 5 Spatial distribution of parameters **a** %Na, **b** PI, **c** KR, **d** RSC, **e** PS

CONCLUSIONS

The Na % values of samples ranged from 8.64 to 63.02 with mean of 33.97, 95 % of samples fall in “excellent” and “permissible” category

- ✓ *Sodium Adsorption Ratio (SAR)* values vary from 0.24 to 11.98 mg/l with a mean of 2.37 mg/l. the majority of samples fall into excellent (95 %) to good (5%) classes similar to Na% classification.
- ✓ The electrical conductivity and SRA values plotted show that the most of the water samples belong into the C2S1class “medium salinity - low sodium” and C3S1 “high salinity - low sodium”, According to the figure, all of the groundwater samples could be utilized for irrigation on practically every soil.
- ✓ Wilcox diagram, reveals that all groundwater samples fall in “excellent ”to “permissible” category except one water sample,
- ✓ *The RSC* values of RSC range from (- 44.30 to - 5.01), these values are less than 1.25 meq/l, the entire water samples fall in good water category, “not hazard ” so suitable for irrigation use
- ✓ *The MH* values range from 11.85 and 48.34 with a mean of 31.19. These values obtained are less than 50 and show that all groundwater samples are “good and suitable” for irrigation
- ✓ *Kelly’s ratio (KR)* values are more than 1 in 85 % of the groundwater samples, the vast majority of samples from the studied area are suitable for irrigation use.
- ✓ *Permeability Index (PI)* values of the samples range from 26.94 to 75.4 with a mean of 52.31. It revealed that, 100 % of water samples fall into “good” water category which indicates that the water is suitable for irrigation purposes
- ✓ *Potential Salinity (PS)* values of groundwater samples range from 1.26 to 80.6 with a mean of 7.64 ; 65% and 15% of the samples were classified as “excellent” to “good”, and were “good” to “injurious” category of water quality respectively.

REFERENCES

Abdourazakou Maman, H., & Arzu, FE. (2022). Statistical assessment of seasonal variation of groundwater quality in Çarşamba coastal plain, Samsun (Turkey). *Environ Monit Assess 194:135. https://doi.org/10.1007/s10661-022-09791-2.*

Barick, BK., & Ratha, SR. (2014). Hydro-chemical analysis and evaluation of groundwater quality of hial area, bolangir district, odisha, india. *journal of geosciences and geomatics. available online at http://pubs.sciepub.com/jgg/2/5a/5*, pp. 5a, 22-28.

Bounab, S., Bousnoubra-kherici, H., Saou, A., & Sedrati, N. (2017). Determination of scaling, corrosion tendencies and water type in the Annaba-el Tarf aquifers, northeastern of Algeria. *j. bio. env. sc 10(5)*, 155-162.

Bouteraa, O., Mebarki, A., Bouaicha, F., Nouaceur, Z., & Laignel, B. (2019). Groundwater quality assessment using multivariate analysis, geostatistical modeling, and water quality index (WQI): a case of study in the Boumerzoug-El Khroub valley of Northeast Algeria. *Acta Geochim 38(6):796–814.* https://doi.org/10.1007/s11631-019-00329-x.

Brown, R. M. (1970). Water quality index-do we dare. *water sewage works, 117(10),* pp. 339-343.

Doneen, L.D. (1964). Notes on water quality in agriculture. *water science and engineering paper 4001, department of water science and engineering, university of california*.

Eaton, F.M. (1950). Significance of carbonate in irrigation waters. *soil science*, pp. 69,127-128.

Gad, M., El-Hendawy, S., Al-Suhaibani,N., Usman Tahir, M., Mubushar,M., & Salah Elsayed, H.S. (2020). Combining hydrogeochemical characterization and a hyperspectral reflectance tool for assessing quality and suitability of two groundwater resources for irrigation in Egypt. *water (mdpi) ,www.mdpi.com/journal/water*.

Horton, R. J. (1965). An index number system for rating water quality. *j. water pollu. cont. fed., 37(3)* , pp. 300-305.

Jhariya. D.C., Kumar, Tarun., Dewangan, Rakesh., Pal, Dharm., Dewangan, Pankaj Kumar. (2017). Assessment of groundwater quality index for drinking purpose in the durg district, Chhattisgarh using geographical information system (gis) and multi-criteria decision analysis (mcda) techniques. *j. geol. soc. india 89, 453. http://dx.doi.org/10.1007/s12594-017-0628-5.*

Karmegam, U., K, *A.*(2010). Study on the mixing proportion in groundwater samples by using piper diagrame and phreeqc model. *Chinese journal of geochemistry 30 (4), 490-495.*

Kawo, Nafyad Serre., & Karuppannan, Shankar. (2018). Groundwater quality assessment using water quality index and gis technique in Modjo river basin, central Ethiopia. *journal of african earth sciences*.

Kelly, W.P.(1963). Use of saline irrigation water. *soil science*, pp. 355-391.

Kelly, W.P.(1940). Permissible composition and concentration of irrigation waters. proceeding*s of the ascf, p 607.*

Kshitindra, KR., Singh, G. T., & Suresh, K. (2020). Evaluation of groundwater quality for suitability of irrigation purposes: a case study in the Udham Singh Nagar, Uttarakhand. *hindawi,journal of chemistry*, p. 15.

Nagaraju, A., Sunil Kumar, K. & Thejaswi, A. (2014). Assessment of groundwater quality for irrigation: a case study from bandalamottu lead mining area, guntur district, Andhra. *appl water science*, pp. 385 - 396.

Oga, M.S., Gnamba, F.M., Adiaffi, B., Soro, T., Oulai, K., & Biemi, J. (2015). Aptitude of groundwaters for irrigation in Katiola area. *Asian review of environmental and earth sciences, vol. 2,*(no.3,), 54-60.

Oulai Jean Gautier, K., Oga, Y. S., Gnamba, FM., & Jean Biemi,Y. S. (2017). Aptitude of ground waters for irrigation in the south-east coastal region of Côted'Ivoire (from abidjanto aboisso). *international journal of engineering science invention, volume 6* (issue 5), pp. 15-20.

Palliwal, KV. (1972). *Irrigation with saline wate no.2.* new delhi, p 198.

Panpan, XU., Wenwen, F., Hui, Q., & Qiying, Z. (2019). Hydrogeochemical characterization and irrigation quality assessment of Shallow groundwater in the central-western Guanzhong basin, China. *international journal of environemental research and public health.*

Portia, Mokoena., Thokozani, Kanyerere., Jan Van, Bever Donker. (2020). Hydrogeochemical characteristics and evaluation of groundwater quality for domestic and irrigation purposes: a case study of the Heuningnes catchment, western cape province, south Africa. *sn applied sciences* . *https://doi.org/10.1007/s42452-020-03339-0.*

Ramya Priya, R., & Elango, L. (2018). Evaluation of geogenic and anthropogenic impacts on spatio-temporal variation in quality of surface water and groundwater along Cauvery river. *India. environ. earth sci. 77 (2). https://doi.org/10.1007/s12665-017- 7176-6.*

Richard, L. (1954). Diagnosis and improvement of saline and alkali soils (vol. 60). *USA,Washington.*

Roder, j. (1996). Chemical and physico-chemical analysis of water; natural water, waste water.

Sadat-Noori, S.M., Ebrahimi, K., & Liaghat, AM. (2014). Groundwater quality assessement using the water quality index and GIS in saveh-nobaran aquifer, Iran. *environ earth sci 71(9): 3827–3843.*

Saleh, A., Al-Ruwih, F., & Shehata, M. (1999). Hydrogeochemical processes operating within the main aquifers of Kuwait. *J Arid Environ* 42:195–209.

Sahu, P., & Sikdar, PK. (2008). Hydrochemical framework of the aquifer in and around east Kolkata wetlands. West Bengal India. *Environ geol 55:823–835*.

Stabler, (1944). A graphic procedure in the geochemical interpretation of water-analyses.

Subbarao, M., & Reddi Bhaskara, R.(2018). Groundwater quality assessment in Srikalahasthi mandal chittoor district, Andhra pradesh, south India. *Iosr journal of engineering (iosrjen). www.iosrjen.org*, pp. 33-42.

Syeda Urooj, F., Moazzam, A K., Farhan ,S., Nadeem, M., Nasir, S., Aamir, A . Syed Shahid, S. (2022). Geospatial assessment of water quality using principal components analysis (PCA) and water quality index (WQI) in Basho Valley, Gilgit Baltistan (Northern Areas of Pakistan). *Environ Monit Assess, 194,151*. *https://doi.org/10.1007/s10661-022-09845-5.*

Szaboles, I., & Darab, C. (1964). The influence of irrigation water of high sodium carbonate content of soils. *proceedings of 8th international congress of international society of soil science (isss), 2,* pp. 803–812.

Tirkey, Poonam., Bhattacharya, Tanushree., & Chakraborty, Sukalyan. (2015). Water quality indices- important tools for water quality assessment. *International journal of advances in chemistry (ijac).*

Wilcox, l. (1955). Classification and use irrigation waters. u.s. departement of agriculture circular, washington,dc.

AGRICULTURAL SUSTAINABILITY AND ECO-FRIENDLY PRACTICES

Furkan Baş[1], Öznur Begüm Gökçek[2], Sevgi Demirel[2]

[1]*Niğde Ömer Halisdemir University Faculty of Agricultural Sciences and Technologies Biosystems Engineering*

[2]*Niğde Ömer Halisdemir University Engineering Faculty Environmental Engineering*

ABSTRACT

According to the Intergovernmental Panel on Climate Change (IPCC) Reports, an increase of 3 to 4 °C in surface average temperature values is expected by 2100 due to the increase in greenhouse gas emissions. Accordingly, a similar temperature increase is expected to be observed in Turkey. This situation, which appears as a global climate crisis, directly affects agricultural production. While sustainable and environmentally friendly production methods are supported with green transformation studies in industry, the implementation of similar approaches in agricultural activities is an important agenda item for the future of sustainable and environmentally friendly agriculture. For this purpose, it is necessary to identify important greenhouse gas sources that occur during agricultural activities and have an impact on global warming. In addition to the chemicals and fertilisers used during agricultural activities, water management is one of the most important focal points of sustainable agriculture. Therefore, adopting sustainable agricultural practices by moving away from traditional agriculture and making these practices a state policy will provide significant benefits in terms of sustainability in the long term. In this study, information is provided on strategies in agricultural systems foradaptation to climate change and technologies required to reduce greenhouse gas emissions. Inaddition, a comprehensive framework for agricultural sustainable development is presented by integrating literature studies in these areas.

Keywords: Sustainable agriculture; climate change; fertiliser management; water management

INTRODUCTION

Climate change is a concept used to refer to changes in long-term weather patterns and can result from both natural processes and human activities. Climate change has been increasing rapidly in recent years, especially due to anthropogenic activities and factors. Among the main causes of human-induced climate change are greenhouse gases released into the atmosphere as a result of intensive combustion of fossil fuels. Among these greenhouse gases, the most important ones are carbon dioxide (CO2), methane (CH4) and nitrous oxide (N2O). Greenhouse gases accumulate in the atmosphere, allowing short-wavelength radiation from the sun to reach the earth, while preventing the long-wavelength radiation reflected from the earth from escaping into space. This process is defined as the greenhouse effect and causes the average surface temperature of the earth to increase (Singh, Delgado-Baquerizo et al. 2023).

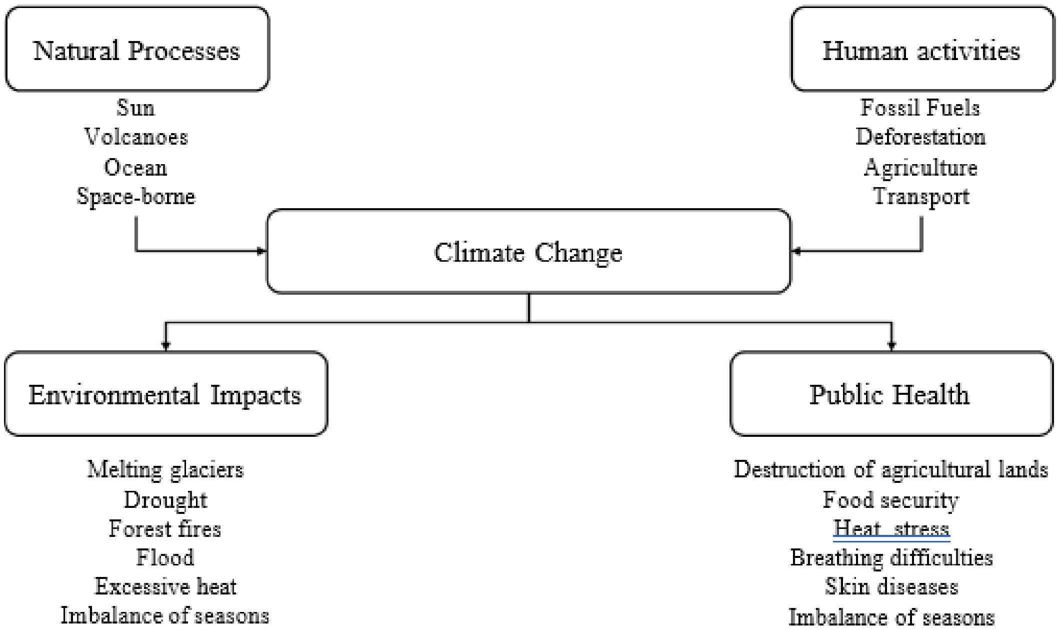


Figure 1. Climate change, causes, and effects.

Human-induced activities such as fossil fuel combustion, energy production, transport and industrial activities are among the main sources of greenhouse gas emissions. In addition, deforestation and destruction of green lands also have a significant impact on climate change. Forests play an important role in the carbon cycle; green plants and trees take CO_2 from the atmosphere through photosynthesis and store it in their biomass. However, the destruction of green areas and forests leads to both the loss of carbon sinks and the release of additional CO_2into the atmosphere as a result of the burning or decay of trees.

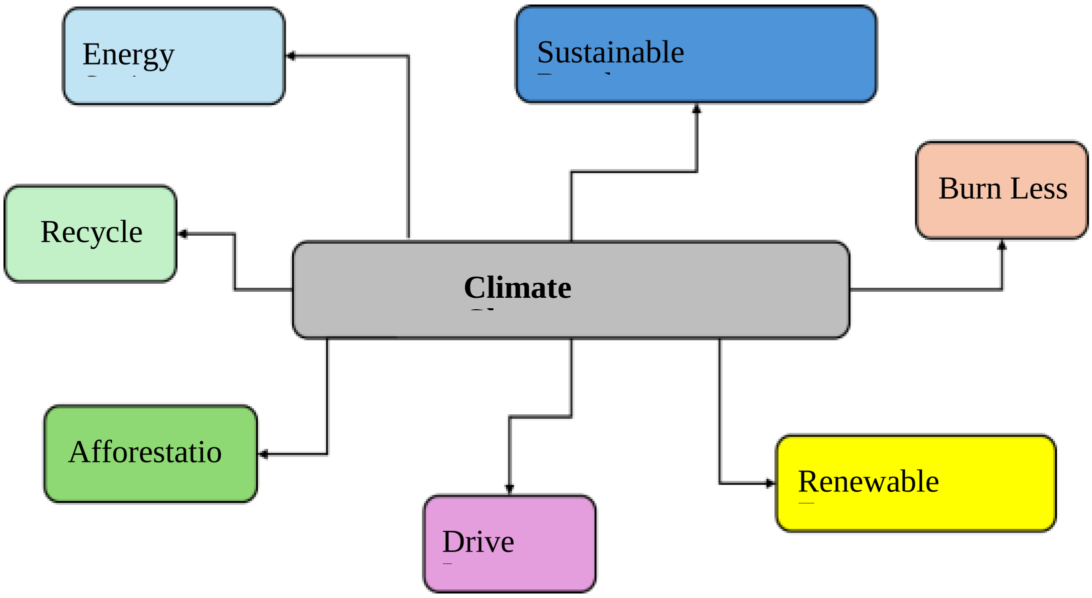


Figure 2. Climate change solutions.

The environmental impacts of climate change can be quite far-reaching. Climate changecan cause many environmental disasters such as global warming, melting of polar ice caps andmountain glaciers, rising sea levels and acidification of oceans. Sea level rise threatens coastalecosystems and human settlements, saltwater intrusion renders agricultural land unproductive and pollutes freshwater resources. Acidification of the oceans causes damage to calcium carbonate organisms (e.g. corals and some plankton species) in marine ecosystems and negatively affects marine biodiversity and fisheries (Louis, Carlson et al. 2023).

Climate change is also increasing the frequency and severity of extreme weather events (e.g. heat waves, severe storms, droughts and floods). These events threaten critical human activities such as agricultural yield-quality and food security. In addition, disasters such as floods can cause direct and indirect damage to human health by destroying infrastructure in settlements. For example, heat waves cause an increase in heat stress and heat-related diseases,while the decrease and deterioration of water resources can trigger the spread of water-borne diseases (Gössling, Neger et al. 2023).

As a result, climate change can have profound and widespread impacts on nature, the environment and ecosystems, as well as on human communities. Reducing greenhouse gas emissions, using renewable energy sources, protecting forests and green areas, sustainable practices and public-private, national-international collaborations are required to overcome thisglobal challenge and end or minimise these impacts. In addition, strategies for adaptation to climate change should be developed and these strategies should be implemented in a sensitive manner.

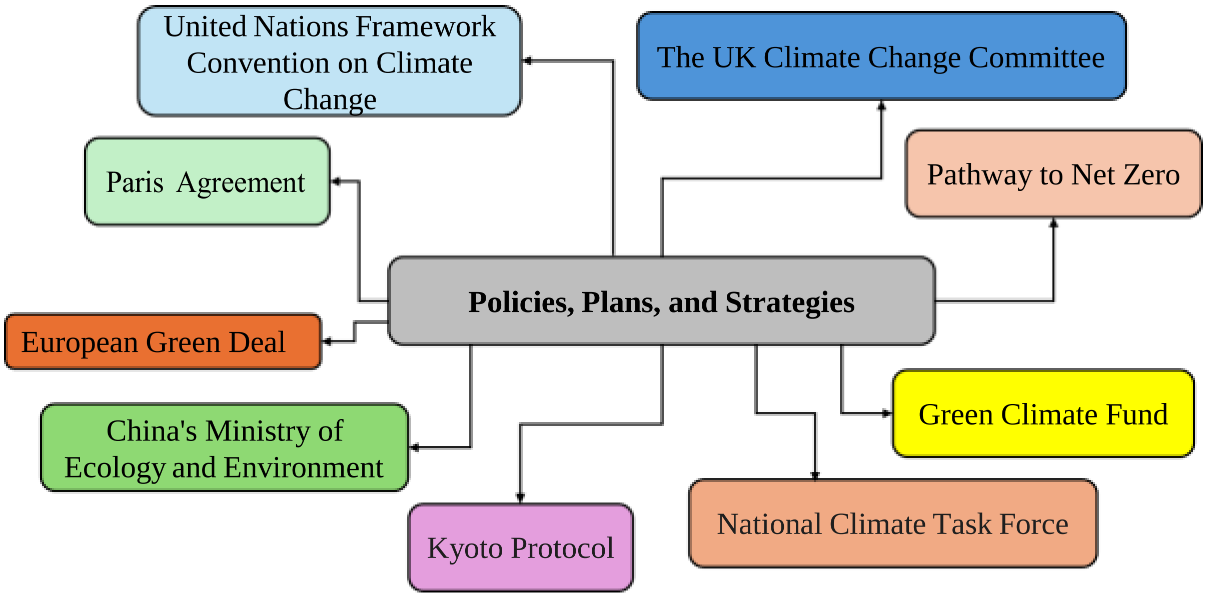


Figure 3. Climate change policies, plans, and strategies.

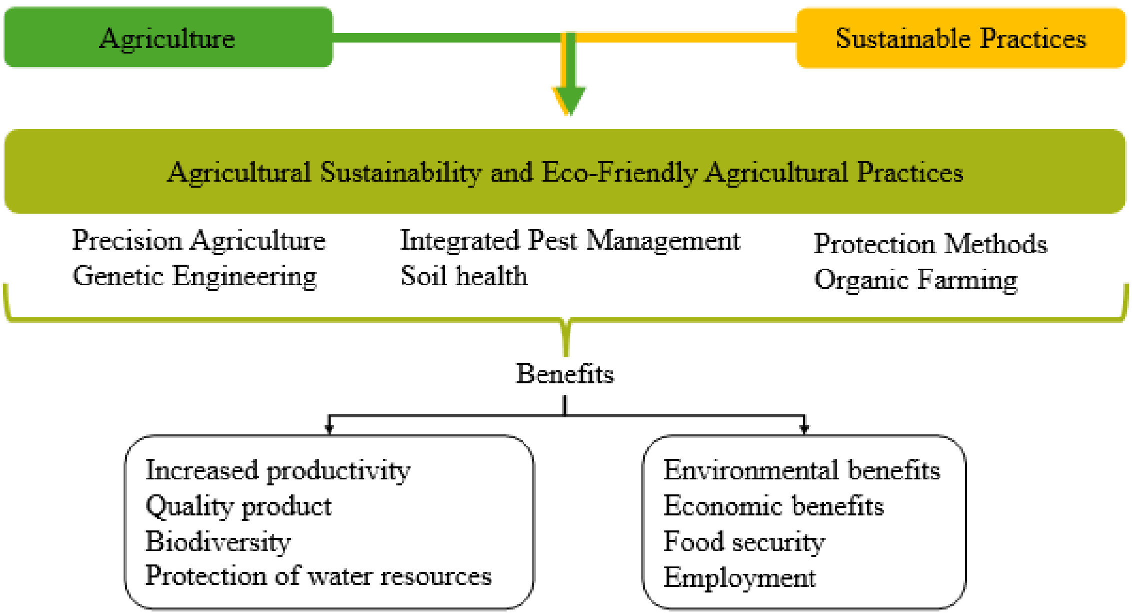


Figure 4. Agriculture and sustainability.

Agriculture also has a critical role in terms of food security and sustainable development. With the rapid increase in the global population, it is imperative to increase agricultural production. However, with the increase in agricultural activities, the environmental impacts of agriculture should also be taken into account. Intensive agricultural practices can lead to many problems such as soil erosion, depletion of water resources and reduction of biodiversity. Therefore, sustainable agricultural practices are important in protecting soil and water resources, maintaining ecosystem services and combating climate change. Alternative agricultural systems such as organic farming, agroecology and permaculture contribute to solving these problems by reducing the use of chemical inputs and increasing biodiversity. Therefore, innovative agricultural technologies and sustainable agricultural practices need to be adopted to increase agricultural productivity. These technologies and practices include precision agriculture, genetic engineering, integrated pest management and soil health protection methods. These practices increase production, yield and quality in agriculture, while at the same time minimising the impacts of agriculture on the environment and the health of living organisms and ensuring that agricultural activities are sustainable (Balasundram, Shamshiri et al. 2023).

RECENT PRACTICES

Rebouh et. al. (2023), investigated that embracing eco-friendly agricultural methods in the wheat farming system holds the potential to engender high and sustainable wheat yields, contingent upon a normative strategy that comprehensively addresses multiple factors. These include the intrinsic attributes of the grown wheat cultivars, plant nutritional parameters, soil agrochemical characteristics, and specific climatic conditions. Further in-depth investigations under field conditions are necessary to help in the discernment of appropriate environmentally agricultural techniques that can efficaciously optimize the yield potential of the different cultivated varieties.

Aroonsrimorakot et al. (2021), conducted a study on Application of Innovative Eco- Friendly Energy Technology for Sustainable Agricultural Farming and examined several technologies that provide sustainability in agriculture. These technologies can be lined as:

- Green Innovative Irrigation
- Sprinkler Irrigation
- Drip Irrigation with Fertilizer (Fertigation System) Technology
- Wind Turbine Energy
- Solar Panel Energy Technology
- Solar Panel Water Pump
- Solar Panel Energy in Agricultural Greenhouse

As a conclusion, they stated that there is an alarming fear of climate change and its impact on the global environment, global population, and the need of using innovative technology in human productive activities for sustainability, including agricultural farming, which is the mainsource of providing food to mankind. Adopting this green energy technology in agricultural farmers has some challenges, which made it to be adopted by only a few farmers and places, due to its high initial investment cost, lack of awareness or training. These difficulties can be overcome or solved through more research studies to optimize the combination of eco-friendlyenergy technology application and agricultural cultivation among the agricultural farmers due to environmental as well as well as economic feasibility (Aroonsrimorakot, Laiphrakpam et al.2021).

Nowak (2021) stated that precision agriculture is a management concept, which relies onintensive data collection and data processing for guiding targeted actions that improve the efficiency, productivity, and sustainability of agricultural operations. In his study, several studies have assessed the adoption rate of precision agriculture technologies at regional or national scale, but the literature lacks global evaluations of the development of precision agriculture. For this paper, a review of 17 papers was conducted to provide an evaluation of the adoption rate of precision agriculture technologies on field crops farms in developed countries.

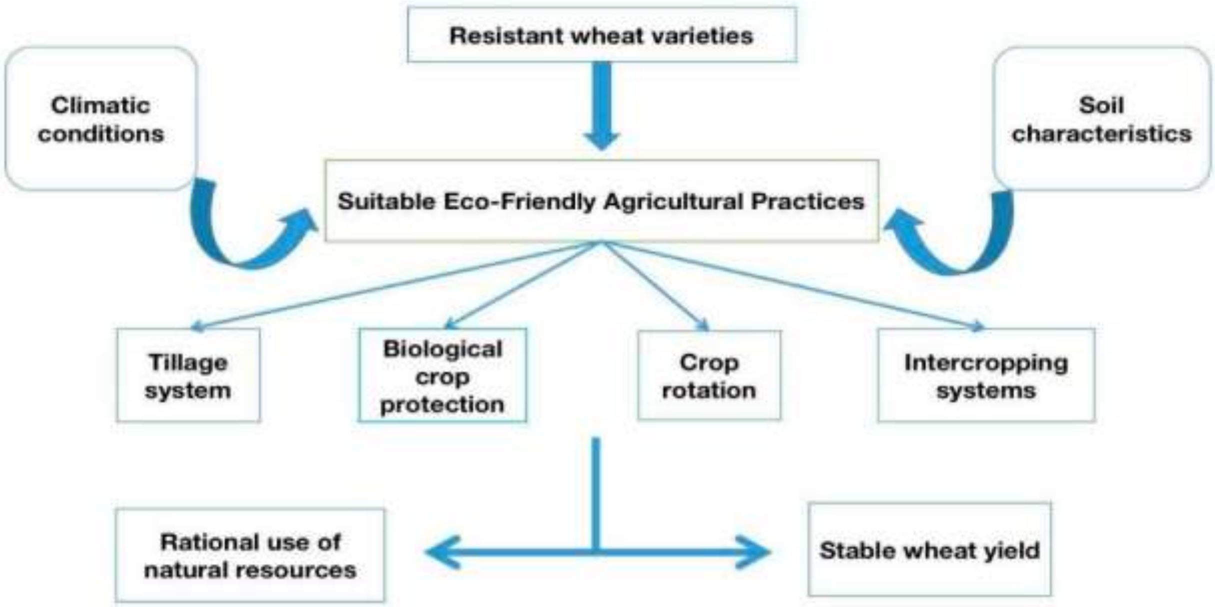


Figure 5. Diagram of integrated wheat farming system according to pedoclimatic conditions(Rebouh, Khugaev et al. 2023).

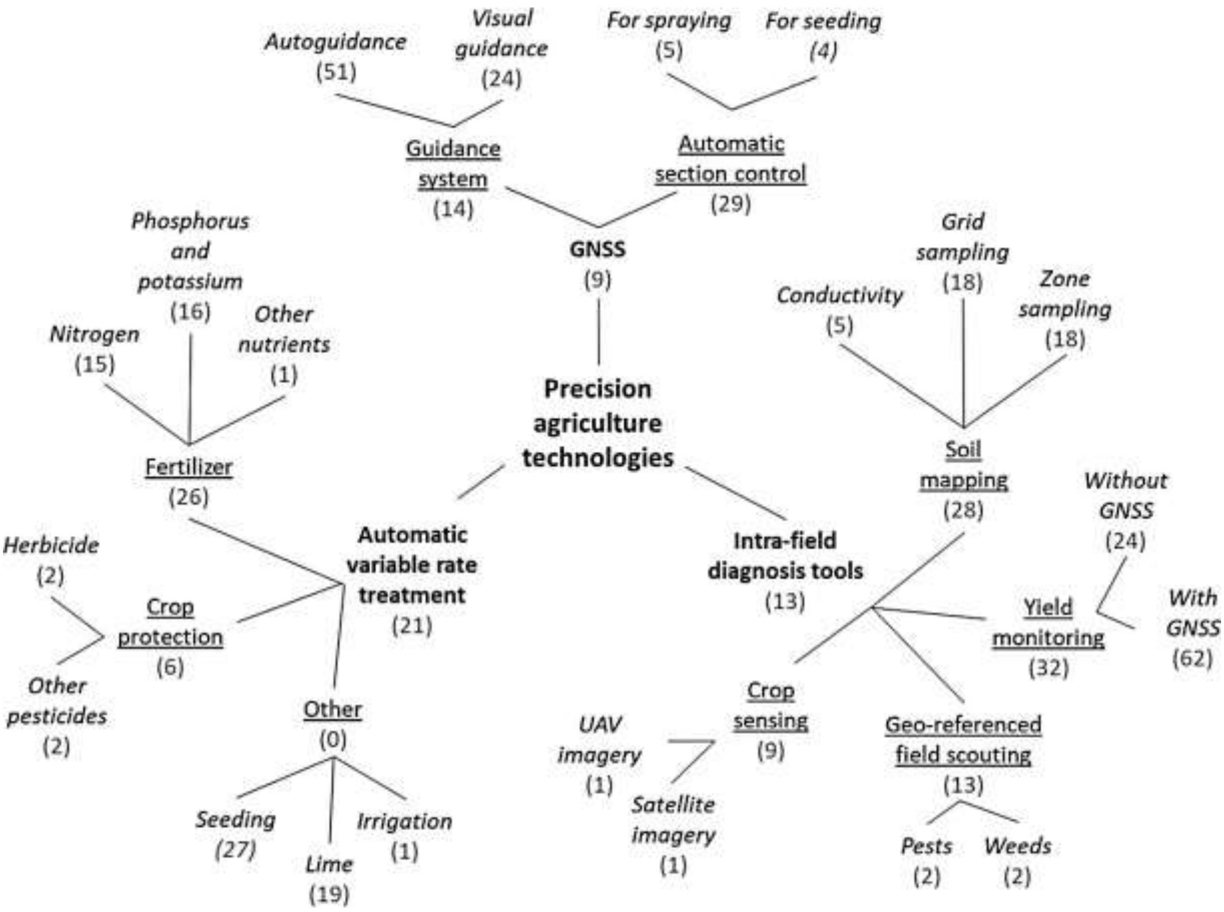


Figure 6. Typology of precision agriculture technologies surveyed in the literature (Nowak 2021).

Hassanisaadi et. al. (2022), investigated that role of agrochemical-based nanomaterials inplants: biotic and abiotic stress with germination improvement of seed. Nanotechnology has provided advancement opportunities in different fields of sciences related to plants such as agriculture. Plants are one of the most critical components of the ecosystem. Therefore, the perception of the behavior of plants in the presence of nanomaterials (NMs) plays an importantrole in achieving the goals of sustainable agriculture. NMs depending on physicochemical andstructural properties show positive or negative effects on plants exposed to them. Additionally,plant effects can be affected differently from species to species. The interaction of the plant with NMs leads to an effect on the morphology and physiology of plant organs. Some NMs play a significant role in improving stresses. The concept of engineered nano-carriers may be apromising route to address difficult challenges in agriculture that could perhaps lead to an increase in crop production while reducing the environmental impact associated with crop protection and food production.

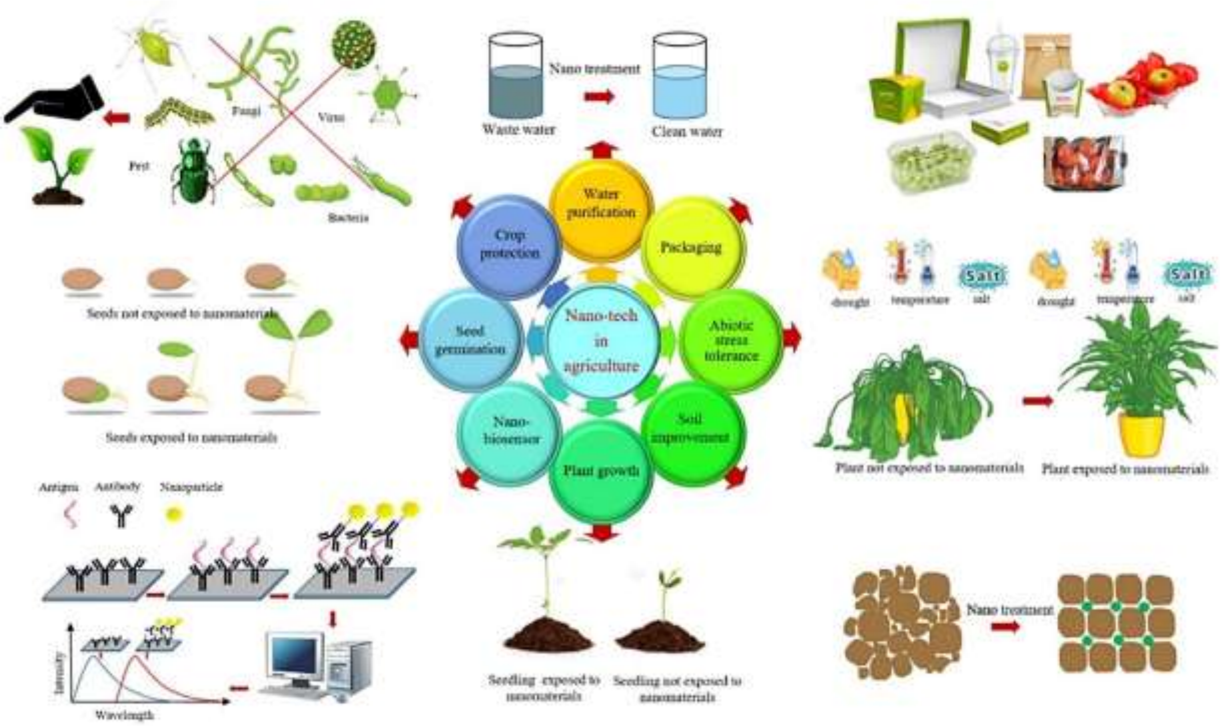


Figure 7. Application of nanomaterials in agriculture (Hassanisaadi, Barani et al. 2022).

CONCLUSION

Agricultural sustainability and eco-friendly practices are critical to ensure that future generations have access to sufficient, healthy and good quality food, water and other vital resources. Widespread adoption of eco-friendly practices such as organic farming, crop rotation,efficient irrigation technologies, eco-friendly fertilisers and the use of renewable energy sourcesin agricultural systems can significantly reduce the footprint of agricultural activities. These practices help maintain soil health, reduce greenhouse gas emissions and protect biodiversity. Modern agricultural practices, such as precision agriculture and sustainable irrigation systems, minimise resource wastage while increasing crop productivity. For a sustainable future in agricultural activities, it is important that farmers, politicians, governments, sectors, and consumers should work together to promote and implement these practices to ensure that farming systems remain resilient and productive without jeopardising the health of our planet.

REFERENCES

Aroonsrimorakot, S., et al. (2021). "Application of Innovative Eco-Friendly Energy Technology for Sustainable Agricultural Farming." Green Technological Innovation for Sustainable Smart Societies: Post Pandemic Era: 211-231.

Balasundram, S. K., et al. (2023). "The role of digital agriculture in mitigating climate change and ensuring food security: An overview." Sustainability **15**(6): 5325.

Gössling, S., et al. (2023). "Weather, climate change, and transport: a review." Natural Hazards **118**(2): 1341-1360.

Hassanisaadi, M., et al. (2022). "Role of agrochemical-based nanomaterials in plants: Biotic and abiotic stress with germination improvement of seeds." Plant Growth Regulation **97**(2): 375-418.

Louis, S., et al. (2023). "Impacts of climate change and air pollution on neurologic health, disease, and practice: a scoping review." Neurology **100**(10): 474-483.

Nowak, B. (2021). "Precision agriculture: Where do we stand? A review of the adoption of precision agriculture technologies on field crops farms in developed countries." Agricultural Research **10**(4): 515-522.

Rebouh, N. Y., et al. (2023). "Contribution of eco-friendly agricultural practices in improving and stabilizing wheat crop yield: A review." Agronomy **13**(9): 2400.

Singh, B. K., et al. (2023). "Climate change impacts on plant pathogens, food security and paths forward." Nature Reviews Microbiology **21**(10): 640-656.

APPLICATION OF OFF-GRID PHOTOVOLTAIC AND PHOTOVOLTAIC/THERMAL SYSTEMS IN EDIRNE-TURKEY

Semiha Öztuna[1], Doğan Eryener[1], Berrin Yılmaz[1], Hacer Akhan[1,], Mustafa Kemalettin Büyükakın[1], Kadir Aydın[1]*

[1] Trakya University, Engineering Faculty, Mechanical Engineering Department, Edirne, Turkey

Corresponding author e-mail: hacera@trakya.edu.tr

ABSTRACT

In photovoltaic systems, the increase in panel temperature is one of the main factors that reduces efficiency. Each 1ºC increase in the temperature of the photovoltaic panel reduces electricity production by 0.45%. In this study, off-grid photovoltaic (PV) and photovoltaic/thermal (PV/T) systems are demonstrated in a low-capacity, low-slope roof application in Edirne, Turkey, and the effect of panel temperature on system performance is investigated by analysing the measurements taken simultaneously under the same environmental conditions. The PV/T system uses an air-heated solar collector with a perforated absorber panel. According to the results of 1 month measurements, in January 2021, the PV system produced 7.9 kWh of electricity and the PV/T system produced 11.3 kWh of electricity. The PV/T system produced 41.5% more electricity. The PV/T system also produced 138.24 kWh of thermal energy.

Keywords: Photovoltaic, Photovoltaic/Thermal, Solar energy, Off-grid

INTRODUCTION

Electricity is the most widely used form of energy in the world today. The fact that electricity generation is heavily dependent on the use of coal, natural gas and other petroleum products raises concerns about the sustainability of production and climate change. With the aim of contributing to the fight against global climate change and the development of renewable energy technologies, the use of renewable energy sources has increased worldwide. In the last 10 years, entirely new concepts in building design and technology have come to the fore and have rapidly spread, especially in the visionary goals of developed countries. Such buil ding designs have been associated with the efficient use of energy and renewable energy technologies that do not harm the environment. In our country, where electricity consumption in residential buildings accounts for more than 20% of total electricity co nsumption, one of the objectives of the Ministry of Energy and Natural Resources has been to use renewable energy sources for micro-generation. As a result of the shift in interest from conventional energy sources to renewable energy, by the end of Februar y 2024, the installed electricity capacity in Turkey was 107,594 MW, of which 54.9% can be supplied by renewable energy sources (Enerji.gov.tr).

Photovoltaic (PV) panels are one of the most common systems used to generate solar electricity from renewable energy sources. However, a systemic disadvantage is that the efficiency of PV panels decreases with increasing temperature. For every 1ºC increase in the temperature of the PV panel, electricity production decreases by 0.45%. Faced with this situation, PV/T systems have been developed to utilise the heat generated in the PV panel to cool it. In PV/T systems, on the one hand, the efficiency is increased by cooling the PV panel and, on the other hand, the thermal energy is recovered.

There are studies on PV and PV/T systems in the literature. Raghuraman established the one-dimensional model of flat plate solar collectors and PV/T collectors to analyse their performance. Finally, Raghuraman presented a number of methods to improve the performance of the collectors (Pattabiraman et al., 1981). Sujala (Sujala et al., 2013), Ruobing (Ruobing et al., 2015) and Jie (Jie et al., 2006) investigated the influence of different characteristics on PV/T collectors. The performance of four configurations of PV/T air collectors has been theoretically investigated by Hegazy (Hegazy, 2020) based on the energy balance concept. Researchers are interested in some of these systems with different end-use applications, including space heating (Sukamongkol et al., 2010) and drying processes (Rajoria et al., 2016). Under Algerian meteorological conditions, Slimani et al. compared three PV/T designs with a PV system (Slimani et al., 2017). Abdul-Ganiyu et al. compared a hybrid PV/T system with a conventional PV system over 25 years in Ghana (Abdul-Ganiyu et al., 2021). Electricity costs are $0.45/kWh for PV and $0.33/kWh for PV/T. Excluding batteries, the conventional PV system was found to be more economical (Abdul - Ganiyu, 2021). Sevela and Olesen studied a PV/T system integrated into buildings (Sevela and Olesen, 2013). By cooling the photovoltaic cells, the PV/T system increased the efficiency of electricity generation by up to 14.8% compared to PV. The PV/T system was found to be more advantageous for use in the Spanish a nd Danish climates (Sevela and Olesen, 2013). Buonomano et al. evaluated the technical and economic feasibility of PV/T collectors compared to conventional PV panels. In the study, an experimental setup consisting of 4 PV systems and 4 polycrystalline silicon PV/T systems with a total power of 2 kW and a surface area of 13 m^2 was constructed. Numerical modelling in TRNSYS simulates the PV/T and PV systems and shows that the PV/T efficiency is about 26%. Experimentally, the average thermal efficiency of PV/T collectors is about 13%, while the electrical efficiency of PV/T and PV systems is 15% (Buonomano et al., 2016). Yuan et al. compared the performance of aqueous PV/T systems, microchannel water tube PV/T systems and conventional PV systems at high ambient temperatures. The experiments showed that the average cell temperatures can reach up to 70°C for aqueous PV/T, 90°C for microchannel PV/T and 100°C for conventional PV module. The electrical efficiency decreased with increasing cell temperature, ranging f rom 11.2% to 10% for aqueous PV/T, 9.6% to 7.7% for micro-channel PV/T and 8.6% to 7.0% for conventional PV (Yuan et al., 2018). Barbu et al. investigated the performance of PV and PV/T systems to meet the growing demand for energy production. An experimen tal prototype system was installed on the campus of the University Politehnica Bucharest. The instantaneous power generation and overall system performance were calculated under four different weather scenarios. The results showed that the performance of the PV/T system is closely related to the effective distribution of the collected thermal energy to the storage tank (Barbu et al., 20 -23).

In this study, a performance analysis of off-grid PV and PV/T systems was conducted. A rooftop demonstration of the systems was set up at the Renewable Energy Systems Test Site of the Faculty of Engineering, Trakya University. The system consists of PV, PV/T, battery, weather station and necessary auxiliary equipment. In the study, a solar air collector with a perforated absorber panel was used as the thermal system. The measurements were used to evaluate both electrical and thermal energy production. The study will be a guide for the application of systems that will produce electricity and heat energy in Edirne and locations with similar climatic conditions.

MATERIAL AND METHOD

Theory

The output power P_{PV} (W) of the PV panel, whose operating voltage is V_{PV} (V) and current I_{PV} (A), is expressed with Equation 1. N_{PV} is the number of PV panels.

$$P_{PV} = V_{PV} \, . \, I_{PV} \, . \, N_{PV} \quad (1)$$

The thermal heat output Q (W) of the air collector is calculated with Equation 2.

$$Q = m \, . \, C_p \, . \, (T_ç - T_g) \qquad (2)$$

In Equation 2, m (kg/s) is the mass flow rate of the air, C_p (J/kg.K) is the specific heating heat of the air, $T_ç$ (ºC) is the air outlet temperature, T_g (ºC) is the air inlet temperature.

PV/T system thermal efficiency is expressed by Equation 3, and electrical efficiency is expressed by Equation 4. G_{rad} (W/m^2) is the solar radiation and $A_{PV/T}$ (m^2) is the collector area.

$$\eta_{th} = [\, m \, . \, C_p \, . \, (T_ç - T_g)]/(A_{PV/T} \, . \, G_{rad}) \qquad (3)$$

$$\eta_e = (V_{PV} \, . \, I_{PV})/(A_{PV/T} \, . \, G_{rad}) \qquad (4)$$

Site characteristics

Edirne is located between 41º-40º north parallel and 26º-34º east meridian (www.edirne.bel.tr). Edirne is about 41 m above sea level and has an area of 6,098 km^2. Due to its location, the city is a transitional zone under the influence of both the Mediterranean climate and the continental climate specific to Central Europe. With temperatures not falling below 30ºC during the summer period, the highest irradiation values per m2 are reached in June, while the longest period of sunshine is observed in July. Figure 1 shows the solar energy potential of Edirne.

Figure 1. Edirne province solar energy potential (solargis.com)

Since the renewable energy source varies according to the geographical structure, the region and field where the power plant is to be installed must be assessed for suitability. Solar radiation, outdoor temperature, wind speed and direction, and outdoor humidity in the installation area were measured by the meteorological station of Trakya University. The individual performances of the system components were determined for the climatic conditions of Edirne at the Renewable Energy Systems Test Site of the Faculty of Engineering, Trakya University, Edirne, Turkey.

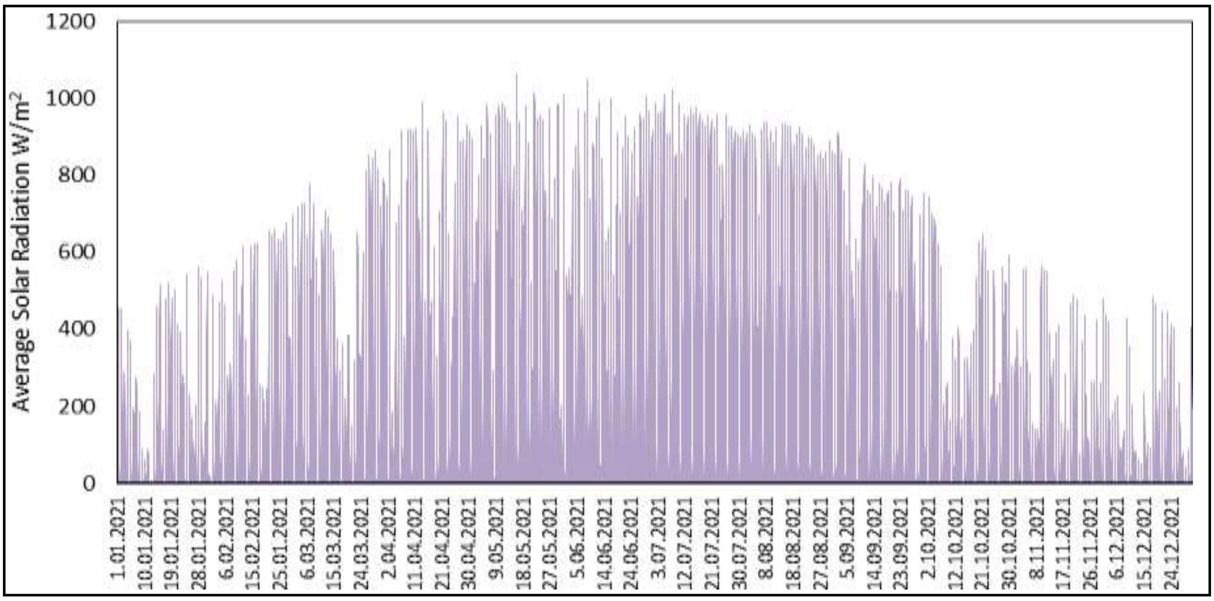


Figure 2. Average hourly solar radiation in Edirne 2021

As can be seen from the measurements in Figure 2, the solar radiation values are extremely good. Solar radiation reaches up to 1000 Watt/m^2 immediately and there is a good potential for PV systems. However, it has been observed that the air temperature reaches up to 40ºC in the summer months, and thus this situation, together with the high radiation, plays an important role in PV heating and reduces the efficiency of electricity generation. In this respect, PV/T systems together have an efficiency-enhancing function.

System characteristics
In order to compare the performance of rooftop PV and PV/T systems, two systems were installed to operate under the same conditions. In the first system installed, the PV panel was mounted on the sandwich panel roof covering. In the second system, the PV panel was tested integrated on a transpired solar air collector. PV panels with the same characteristics were used in both systems. An important component of the PV/T system is the solar air collector with perforated absorber panel. This is a façade cladding system made of metal material that is normally installed on the outside of buildings. The TSC is used for space heating in residential buildings, space heating and production of hot process air in industrial plants. The main element of the system is the perforated absorber panel, which absorbs solar energy. The absorber panel heats up because of solar radiation and, thanks to its many specially designed millimetre-sized holes, heats the outside air and sends it into the room. Due to this structure, the collector is used as one of the ideal systems for PV/T applications.

Both systems are positioned on the frame structure, facing south, simulating a 10 º roof pitch. The PV and PV/T system elements and characteristics are shown in Table 1, the PV and PV/T system schematics are shown in Figure 3, the PV and PV/T systems are shown in Figure 4, the PV/T system fan and data logger are shown in Figure 5. The inverter and batteries are shown in Figure 6, the data logger and charge controllers in Figure 7, and the LED projectors as power consumers in Figure 8. Table 2 shows the technical specifications of the PV panels. PV panels with the same characteristics were used in both systems. Table 3 shows the technical specifications of the solar air collector and Table 4 shows the specifications of the fan. Figure 9 shows the temperature sensors mounted on the PV system and Figure 10 shows the temperature sensors mounted on the PV/T system.

Table 1. PV and PV/T system components and specifications

System Components	**Specification**
Photovoltaic (PV) system	
Polycrystalline solar panel	280 W, 24 V
Solar Charge Controller	30 A
Photovoltaic/Thermal (PV/T) system	
Polycrystalline solar panel	280 W, 24 V
Transpired Solar Collector	5 m^2
Fan	
Solar Charge Controller	30 A
Fan speed controller	
Other equipment	
Battery	100 ah, 12 V
Inverter	1200 W, 24 V
AC led floodlights (Resistance, load)	150 W
Data Loggers	
Measurement system	

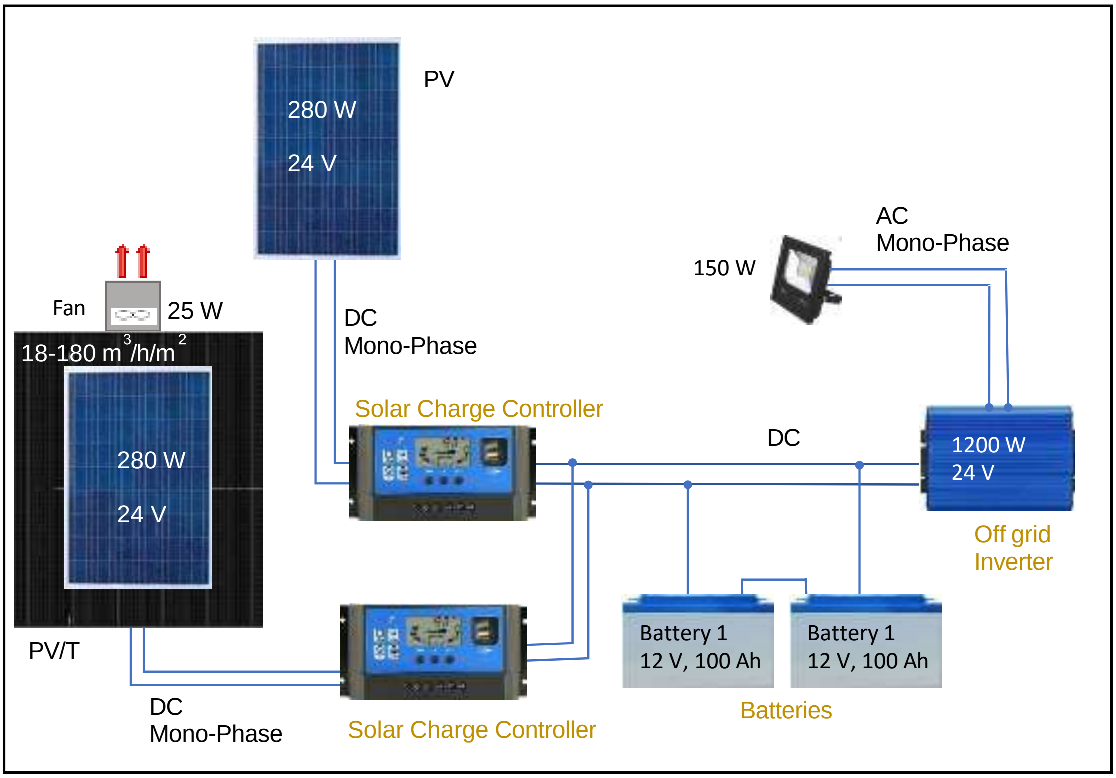


Figure 3. Schematic drawing of PV and PV/T systems

a) b)

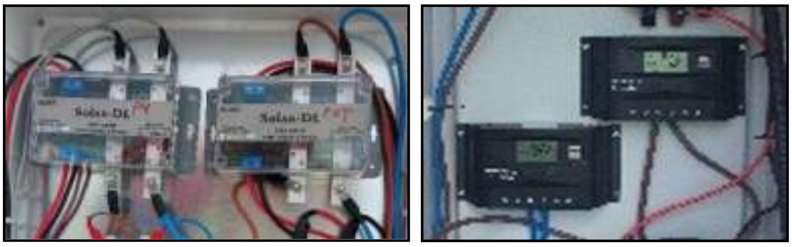
Figure 4. a) PV and b) PV/T systems

a) b)

Figure 5. PV/T system fan and data logger

a) b)

Figure 6. a) Inverter and b) Batteries



a) b)

Figure 7. Data logger and charge controllers

Figure 8. Power consumer led projectors

Table 2. PV panel technical specifications

Specification	
Cell type	Polycrystalline
Cell arrangement	60 (60x1)
Size	1640x990x35 mm
Weight	19 kg
Front coating	3,2 mm tempered glass
Frame material	Alloy aluminum
J-box	IP67
Cable	4 mm^2 (IEC)/12AWG(UL), 900 mm
Connector	MC4
Nominal Max. Power	280 W
Optimum Operating Voltage	31,2 V
Optimum Working Current	8,97 A
Open Loop Voltage (Voc)	37,8 V
Short Cycle Current (Isc)	9,50 A
Module efficiency	%19
Operating temperature	-40ºC~+85ºC
Application Class	A
Power Tolerance	0 ~ 5 W

Table 3. Technical specifications of solar air collector

Specification	**Value**
Collector absorptivity	0,92
Collector area	5 m^2
Collector orientation	0º azimuth
Air flowrate max (per 1m^2)	180 m^3/h /m^2

Table 4. Fan specifications

Specification	**Value**
Voltage	230 V/50 Hz mono-phase
Max. Power	15 W
Max. Air Flowrate	295 m^3/h
Certificate	IPX4, EU 1253/2014 and ERP 2009/125/CE

Figure 9. Temperature sensors installed on the PV system

Figure 10. Temperature sensors installed on the PV/T system

The current, voltage and temperature values of both systems were measured and recorded simultaneously. 7 temperature sensors were installed on the systems. The temperature sensors were connected to data recorders and the readings were recorded.

Solar radiation, outdoor temperature, wind speed and direction, and outdoor humidity in the installation area were measured continuously, 24 hours a day, 7 days a week, in all weather conditions by the meteorological station of Trakya University. Theweather station is shown in Figure

Figure 11. Trakya University meteorology station

RESULTS AND DISCUSSION

The energy production of the PV and PV/T systems was measured and recorded simultaneously. Using the data measured under the same climatic and operating conditions when the systems were operating simultaneously, the energy production, power profiles and system performances for PV and PV/T systems were determined and their performances were compared.

On 8 January 2021, Figure 12 shows the air temperature and solar radiation, Figure 13 shows the current and voltage values of the PV and PV/T systems, and Figure 14 shows the power production diagrams of the systems. As can be seen from the graph, the PV/T system produced 29% more power than the PV system.

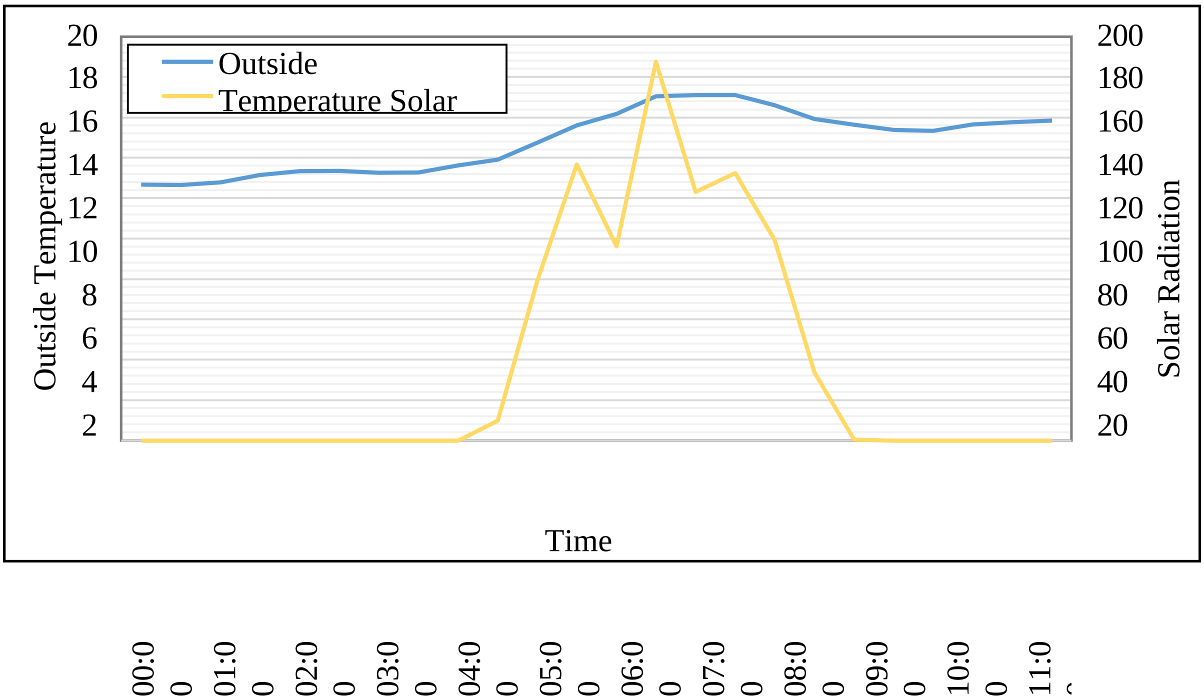


Figure 12. Air temperature and solar radiation on 8 January 2021

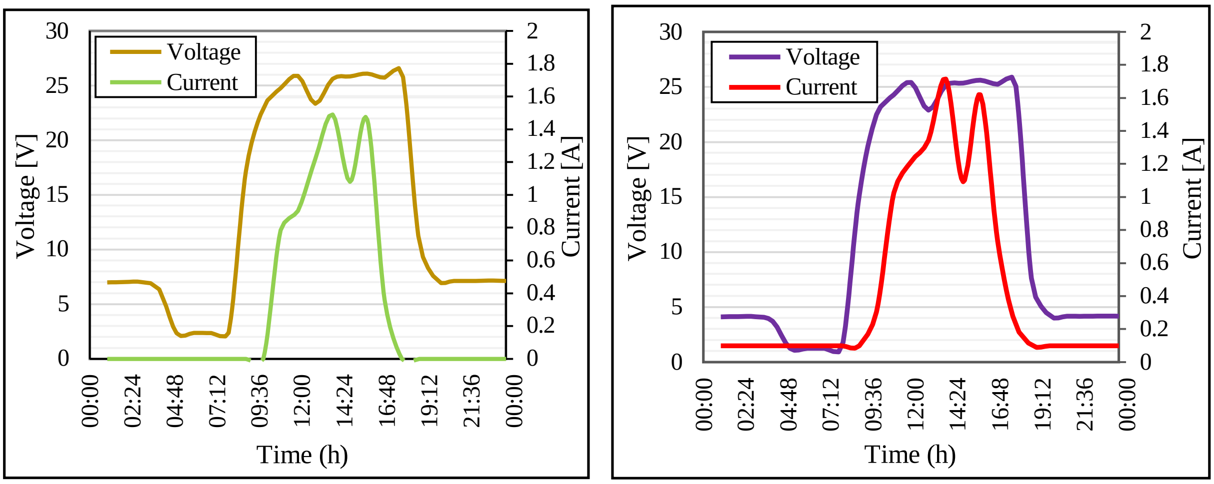


Figure 13. PV and PV/T systems current and voltage values on 8 January 2021

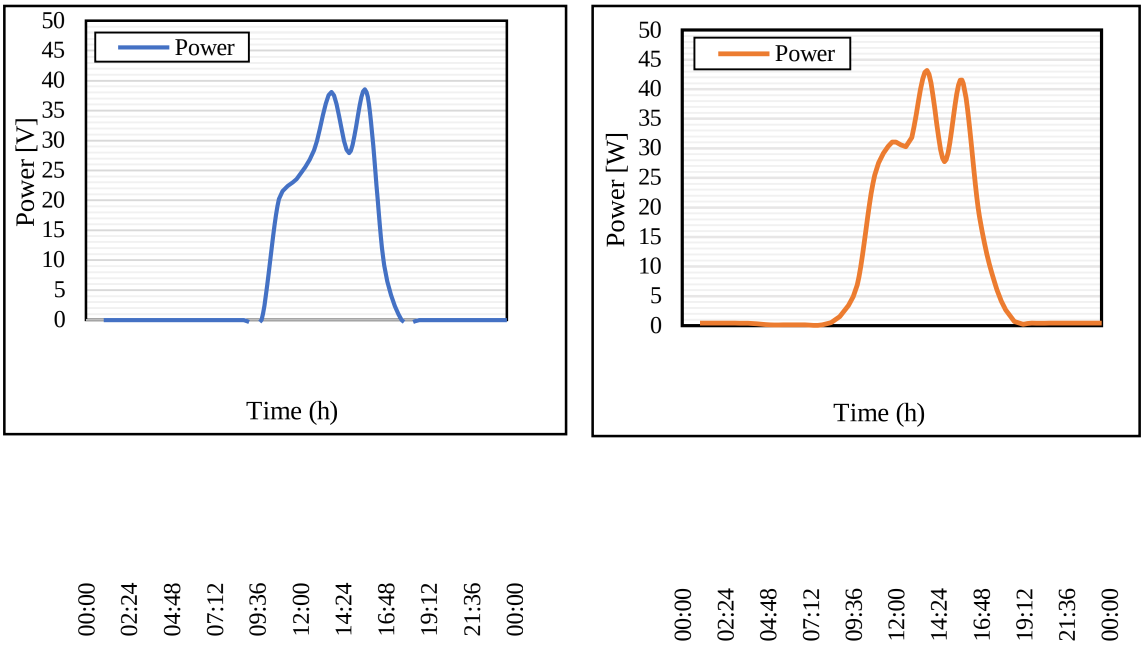


Figure 14. PV and PV/T systems power generation on 8 January 2021

Figure 15 shows the electricity production values of the PV and PV/T systems in January 2021. Figure 16 shows the thermal power distribution produced simultaneously by the PV/T system. The difference in panel temperatures reached 2.2 ºC in January 2020. A very important issue in cooling is the effect of the fan flow rate used. Normally, the cooling efficiency increases as the flow rate increases, but this effect was not investigated during the experiments. The system was operated at a constant flow rate because there are many parameters in the system and some basic functions were kept constant to study them effectively. In the future, the change in cooling efficiency for different flow rates will be investigated. Figure 17 shows the solar radiation data and efficiency comparison of the PV and PV/T systems.

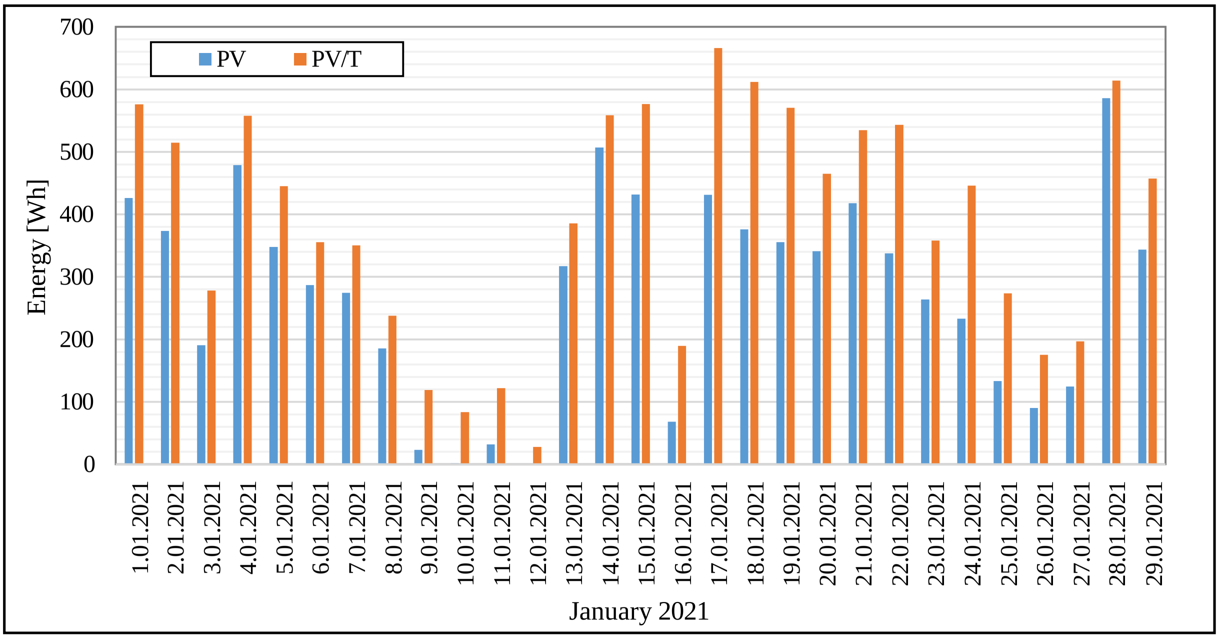


Figure 15. PV and PV/T systems electricity production in January 2021

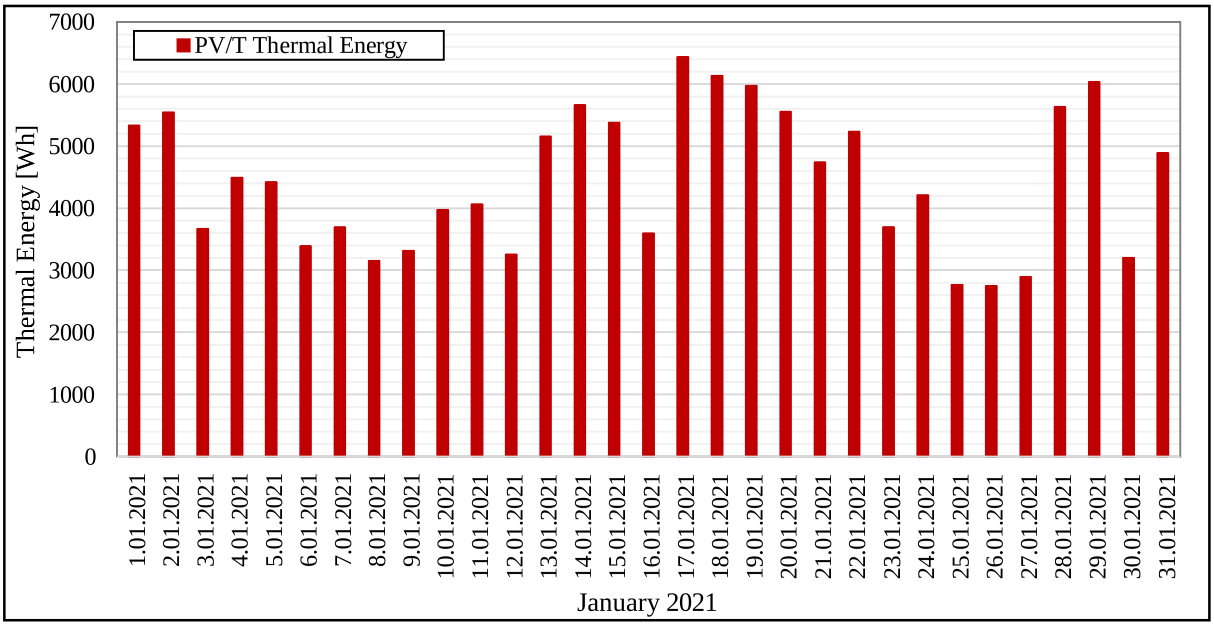


Figure 16. PV/T system thermal power production in January 2021

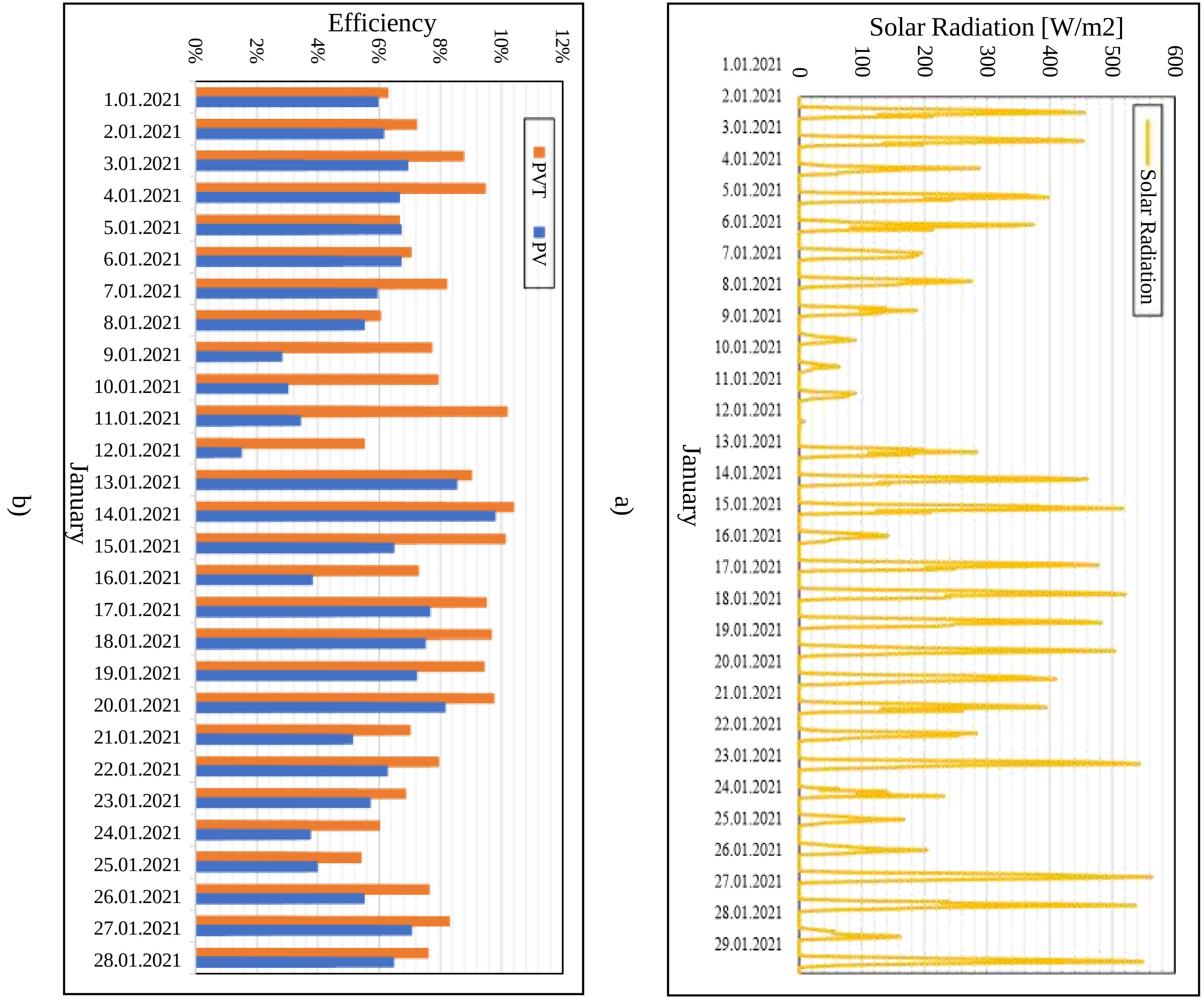


Figure 17. a) Solar radiation data in January 2021 b) Efficiency comparison of PV and PV/T systems

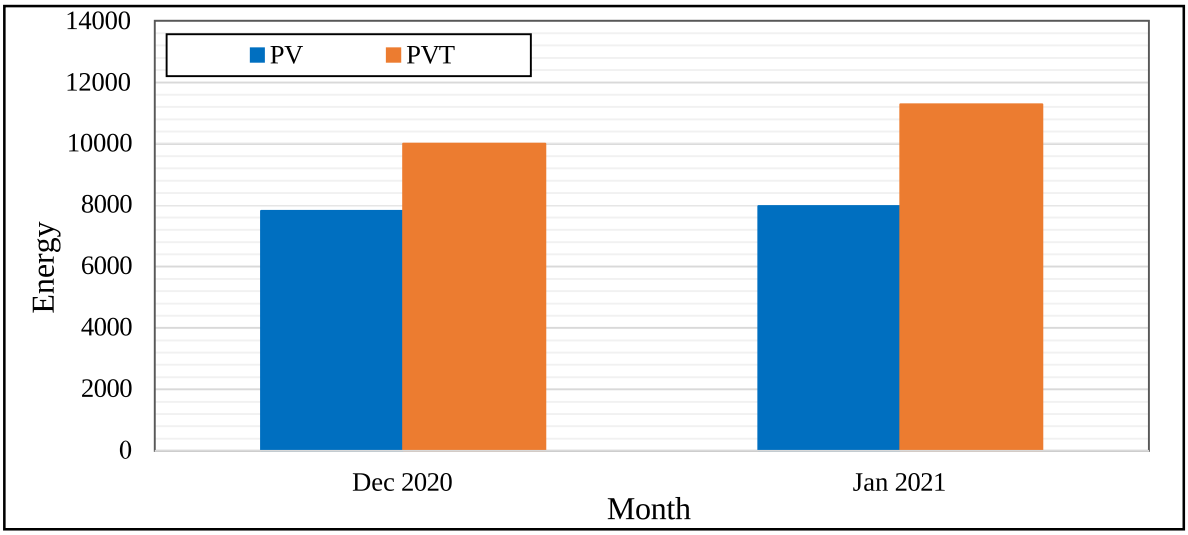


Figure 18. Total electricity production from PV and PV/T systems in December 2020 and January 2021

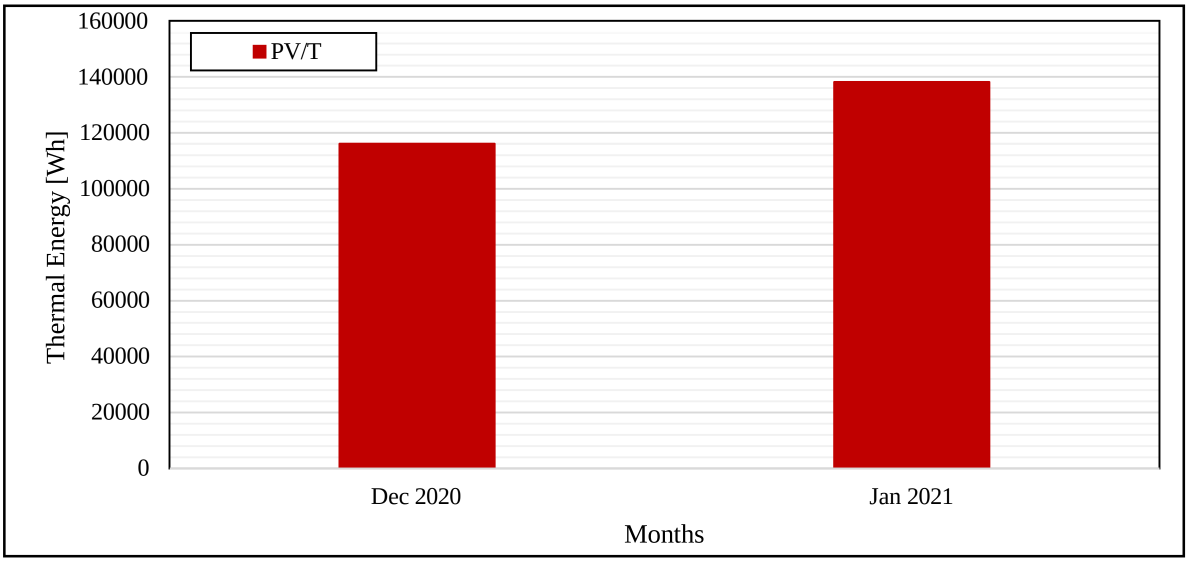


Figure 19. Total thermal energy production from PV/T systems in December 2020 and January 2021

Figure 18 and Figure 19 show the comparison of the total energy production of the PV and PV/T systems in December 2020 and January 2021. In December, the thermal energy produced by the thermal collector of the PV/T system is 116,095.7 Wh and the electrical energy is 7,812.8 Wh, and the electrical energy produced by the PV system is 10,000.2 Wh. In January, the thermal energy produced by the thermal collector of the PV/T system is 138,247.4 Wh and the electrical energy is 11,291.4 Wh, and the electrical energy produced by the PV system is 7,976.1 Wh. Comparing the energy production in January and December, the PV system produced 2% more electrical energy and the PV/T system produced 12% more electrical energy and 19% more thermal energy in January.

Looking at the energy production of the hybrid system, the PV/T system produces 41.5% more electricity than the PV system in January 2021, as expected. While the electrical energy produced by the cooled panel in the PV/T system increases significantly, the heat extracted from the PV panel is converted into useful energy.

CONCLUSIONS

The project evaluated the performance of Edirne province for off-grid applications of different solar energy technologies. In the analyses carried out within the framework of the project, the electricity production in small-scale production applications in Edirne province was determined to be 7,976.1 Wh for PV/T and 11,291.4 Wh for PV. At the same time, 138,247.4 Wh of thermal energy was produced with the PV/T system. Based on the analysis results, it appears that PV/T systems are more suitable to meet the electricity demand of buildings in Edirne and similar climatic conditions. In January 2021, the electricity production of the PV/T system was 41.5% higher than that of the PV system. Operating conditions, i.e. continuity at the point of consumption, also play a decisive role.

ACKNOWLEDGEMENT

This study was funded by Trakya University Scientific Research Projects Unit (TÜBAP) (Project No: 2020/31).

REFERENCES

Abdul-Ganiyu, S., Quansah, D. A., Ramde, E. W., Seidu, R., Adaramola, M. S. 2021. Techno- economic analysis of solar photovoltaic (PV) and solar photovoltaic thermal (PVT) systems using exergy analysis. Sustainable Energy Technologies and Assessments. 47: 101520.

Barbu, M., Siroux, M., Darie, G. 2023. Performance Analysis and Comparison of an Experimental Hybrid PV, PVT and Solar Thermal System Installed in a Preschool in Bucharest, Romania. Energies. 16(14) : 5321.

Buonomano, A., Calise, F., Vicidomini, M. 2016. Design, simulation and experimental investigation of a solar system based on PV panels and PVT collectors. Energies. *9*(7): 497.

Hegazy AA. 2000. Comparative study of the performances of four photovoltaic/ thermal solar air collectors. Energy Convers Manage. 41(8): 861–81.

Jie, J. 2006. Effect of fluid flow and packing factor on energy performance of a wall-mounted hybrid photovoltaic/water-heating collector system. Energy Build. 38(12):1380-7.

Pattabiraman, R. 1981. Analytical predictions of liquid and air photovoltaic/thermal, flat-plate collector performance. J Sol Energy Eng. 103(4): 291-8.

Rajoria CS, Agrawal S, Chandra S, Tiwari GN, Chauhan DS. 2016. A Novel investigation of building integrated photovoltaic thermal (BiPVT) system: a comparative study. Sol Energy. 131: 107–18.

Ruobing, L. 2015. Performance evaluation of new type hybrid photovoltaic/ thermal solar collector by experimental study. Appl Therm Eng. 75: 487-92.

Sevela, P., Olesen, B. W. 2013. Development and benefits of using PVT compared to PV. Sustainable Building Technologies. 90-97.

Slimani MEA, Amirat M, Kurucz I, Bahria S, Hamidat A, Chaouch WB. 2017. A detailed thermal-electrical model of three photovoltaic/thermal (PV/T) hybrid air collectors and photovoltaic (PV) module: Comparative study under Algiers climatic conditions. Energy Convers Manag. 133: 458–76.

Sujala, B. 2013. Comparative study of photovoltaic and thermal solar systems with different storage capacities: performance evaluation and economic analysis. Energy. 61: 272-82.

Sukamongkol Y, Chungpaibulpatana S, Limmeechokchai B, Sripadungtham P. 2010. Condenser heat recovery with a PV/T air heating collector to regenerate desiccant for reducing energy use of an air conditioning room. Energy Build. 42: 315–25.

Yuan, W., Ji, J., Li, Z., Zhou, F., Ren, X., Zhao, X., Liu, S. 2018. Comparison study of the performance of two kinds of photovoltaic/thermal (PV/T) systems and a PV module at high ambient temperature. Energy. 148: 1153-1161.

https://enerji.gov.tr/bilgi-merkezi-enerji-elektrik

https://solargis2-web-assets.s3.eu-west-1.amazonaws.com/public/flyers/3ce9214b1a/Solargis_maps-2019-04-26_EU-EN-web.pdf

COMPARATIVE ANALYSIS OF HORIZONTAL AND VERTICAL AXIS WIND TURBINES IN EDIRNE-TURKEY

Semiha Öztuna[1], Doğan Eryener[1], Berrin Yılmaz[1], Hacer Akhan[1,], Mustafa Kemalettin Büyükakın[1], Kadir Aydın[1]*

[1] Trakya University, Engineering Faculty, Mechanical Engineering Department, Edirne, Turkey

Corresponding author e-mail: hacera@trakya.edu.tr

ABSTRACT

In Turkey, where residential electricity consumption accounts for more than 20% of the total electricity consumption, it is becoming increasingly important to use renewable energy sources for micro-scale electricity generation. In this study, two different micro-scale wind power generation systems that can be integrated into buildings have been installed. The performance of these systems is investigated theoretically and experimentally for real field conditions. As part of the study, a hybrid system consisting of a horizontal axis wind turbine and a vertical axis wind turbine with electricity storage and grid connection was installed at the Renewable Energy Systems Test Site of the Faculty of Engineering, Trakya University, and the electricity production of these systems was monitored for a period of two years. The data obtained was used to determine system performance and energy production. According to the measurement and analysis results, the HAWT system produced 153% more electricity in December 2020 and 168% more electricity in January 2021 than the VAWT system.

Keywords: Horizontal axis wind turbine, Vertical axis wind turbine, Wind energy, Off-grid

INTRODUCTION

As in the rest of the world, electricity consumption in Turkey is increasing day by day. Electricity consumption increases almost every year due to many factors such as population growth, widespread use of electrical appliances and technology products, and industrial growth. In 2023, Turkey's electricity consumption will decrease by 0.2% year-on-year to 330.3 TWh and electricity generation will decrease by 0.6% year-on-year to 326.3 TWh. In 2023, 36.3% of our electricity generation came from coal, 21.4% from natural gas, 19.6% from hydropower, 10.4% from wind, 5.7% from solar, 3.4% from geothermal and 3.2% from other sources. At the end of February 2024, Turkey's installed capacity reached 107,594 MW. At the end of February 2024, the breakdown of installed capacity by source is as follows: 29.7% hydro, 23.3% natural gas, 20.3% coal, 11.1% wind, 11.5% solar, 1.6% geothermal and 2.5% other sources. By the end of June 2022, the installed capacity based on wind energy was 10,976 MW, representing 10.81% of the total installed capacity. According to the results of Turkey's National Energy Plan, electricity consumption is expected to reach 380.2 TWh in 2025, 455.3 TWh in 2030 and 510.5 TWh in 2035 (enerji.gov.tr). In our country, the production of electricity using renewable energy sources is one of the goals of the Ministry of Energy and Natural Resources. At this stage, wind turbines used in wind power generation are becoming more and more important.

The most important studies on wind turbines in recent years: Zhao et al. studied the optimal geometric configurations for performance enhancement of vertical axis wind turbines (Zhao et al., 2006). In order to determine the turbine performance, parameters such as blade load and average rotor torque coefficient were obtained for different configuration cases. From the numerical studies, an increase of up to 23% could be calculated for the vertical axis wind turbine pair using a deflector. Kouloumpis et al. evaluated the potential of small-scale vertical axis wind turbines (VAWT) as a solution to climate change (Kouloumpis et al., 2020). A real example of a 5 kW H-Rotor Darrieus VAWT in Poland was studied for performance using real production data. Eltayesh et al. conducted experimental and numerical studies on a small horizontal wind turbine (Eltayesh et al., 2021). The effect of the number of blades on the turbine power and thrust coefficient was investigated. The results show that the power coefficient increases as the number of blades decreases. Zhao et al. review existing approaches to aerodynamic performance from the perspective of geometric parameters, flow control methods, blade shape modification, power boosting devices, hybrid systems and variable pitch control to further understand the performance improvement of vertical wind turbines (Zhao et al., 2022). In addition, several studies on the performance improvement of vertical wind turbines are summarised. Borg et al. investigated vertical wind turbines compared to horizontal wind turbines (Borg et al., 2014). It was concluded that vertical wind turbines had many advantages. These were siting, increased capacity and scale, challenging conditions and installation factor. Peng et al. review recent research on the turbulent aerodynamics of H-rotor vertical wind turbines (Peng et al., 2021). The results of these studies show that the turbulent motions generated in vertical wind turbines are characterised by features such as strong asymmetry and counter-rotating vortex motion. In addition, this study provides recommendations for the study of vortex and turbulent motions in vertical wind turbines. Pourrajabian et al. studied horizontal wind turbine blades using genetic algorithms (Pourrajabian et al., 2021). The study investigates the accuracy and precision of two different genetic algorithms for the wind turbine blade optimisation problem. The results show that the continuous genetic algorithm outperforms the binary one in terms of accuracy and computation time.

Comparative studies are important to determine which type of wind turbine has the higher potential. There are comparative studies in the literature on Horizontal Axis Wind Turbine (HAWT) and Vertical Axis Wind Turbine (VAWT) systems. In their study, Johari et al. compared the performance of two different designs to build a functional wind turbine and under the behaviour of wind at different speeds. They highlighted that the HAWT is able to generate higher voltage, but the voltage drops to zero when the wind angle changes. However, they observed that the VAWT generates lower voltage, but changes in wind angle do not affect the voltage output at all (Johari et al., 2018). Paraschivoiu et al. compared the aerodynamic performance and feasibility of multi-megawatt VAWTs with existing horizontal axis wind turbines of similar power rating. Two different types of three-blades VAWTs were designed: one with a Darrieus (H-type) and the other with a Troposkien (Φ-type) rotor configuration, in the rated power range of 2, 4 and 6 MW, based on an optimal parametric study. This comparative performance study showed that VAWTs have a simple design, which is not only characterised by superior performance, but also highly economical in terms of manufacturing, maintenance and cost (Paraschivoiu et al., 2018). Eftekhari et al. made a general comparison between HAWTs and VAWTs.

They also investigated the aerodynamic performance of newer VAWT designs. This comparative study showed that for large-scale VAWTs, three-bladed turbines positioned upwind are the most suitable option (Eftekhari et al., 2022). Fadil et al. investigated the effect of wind speed variations on the power output of wind turbines. They used an H-Darrieus type VAWT and a HAWT of the same size. Both turbines have three blades and NACA 4412 airfoils. The results showed that the CP of th e HAWT was 0.54 and the maximum power output was 1363.6 W, while the CP of the VAWT was 0.34 and the maximum power output was 505.69 W (Fadil and Ashari, 2017). Al-Rawajfeh and Gomaa made a comparison between VAWTs and HAWTs, emphasising the importance of both designs. HAWTs are considered more suitable in remote and offshore areas for large capacities with efficiencies up to 50%, while VAWTs are generally more suitable for smaller, urban and offshore installations with efficiencies around 40% (Al-Rawajfeh and Gomaa, 2017).

In this study, a comparative analysis of off-grid horizontal and vertical axis wind turbine systems is carried out. The application of both systems has been installed at the Renewable Energy Systems Test Site of the Faculty of Engineering of Trakya University. Both turbines are operated simultaneously, and their performance is compared under the same climatic and operating conditions. In this respect, it has been determined which type of turbine is more effective for small -scale energy production in Edirne's climatic conditions, for example for meeting domestic energy needs, under real field conditions. With the start of field measurements, both theoretical performance calculations and actual performances were measured, and the real efficiencies and capacity factors of each system were determined. The measurements were used to evaluate the electrical energy production. It was determined which system would have a higher performance in the climatic conditions of Edirne and proved with the analyses made with the data measured because of two years of experimental studies. The study will be a guide for the applications of wind energy systems that will produce electrical energy in Edirne and similar climatic conditions.

MATERIAL AND METHOD

Theory

This section presents the wind turbine power calculations of the hybrid power generation system. Considering that a wind turbine is a machine that rotates by momentum exchange of air mass particles flowing through the blade sweep area, the wind power in the sweep area A (m^2) is proportional to the cube of the size of this area, ρ (kg/m^3) the density of air and VH (m/s) the wind speed. Accordingly, the wind power PT (W) is expressed by Equation 1 (Simmons, 1975).

$$P = 1 \rho A V^{3} \qquad (1)$$

T ‾2 H

The wind turbine output power *PT* is expressed by Equation 2. Turbine operating voltage *VT* (V) and current *IT* (A) are expressed by the symbols.

$$P_T = V_T \, x \, I_T \quad (2)$$

Characteristics of the installation location

Since the renewable energy source varies according to the geographical structure, the location where the energy generation system is to be installed should be evaluated in terms of its suitability. Figure 1 shows the wind energy potential of Edirne provinc e. Thrace is a region with high wind potential. In the provinces of Tekirdağ, Kırklareli and Edirne, a total area of 2,234 km ^{2} is suitable for the installation of a wind energy system and a power generation plant with a capacity of 11,175 MW can be install ed. In addition, the provinces are suitable for the installation of solar energy systems with an average solar radiation of 1,400 -1,450 kWh/m^2.year (Thrace Development Agency, 2012). Solar radiation, outdoor temperature, wind speed and direction, and outdoor humidity were measured at the installation site with the meteorological station of Trakya University.

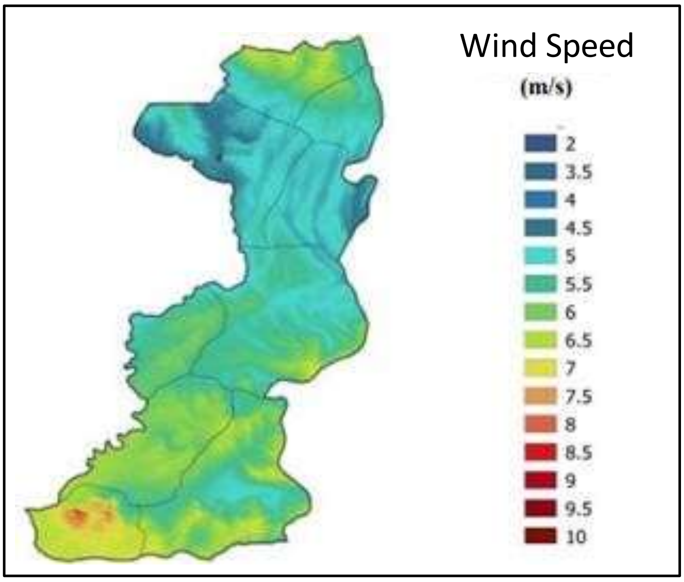


Figure 1. Edirne province wind energy potential (100 m) (repa.enerji.gov.tr)

System Specifications

In the study, an application consisting of a 500 W vertical axis wind turbine and a 500 W horizontal axis wind turbine was created. The application used two 12 V, 100 Ah batteries and a 24 V, 1200 W inverter. A timer relay socket was used for automation and the 50 W power consuming element was operated for 6 hours per day to ensure consumption. Table 1 shows the wind energy system elements and their technical specifications. Figure 2 shows a schematic drawing of the HAWT and VAWT systems and Figure 3 shows photographs of the system components.

Table 1. Wind energy system elements and their technical specifications

System Element	Specifications
Vertical axis wind turbine system	
Vertical axis wind turbine	500 W, 12 V, 30 A
Charge Controller	24 V
Horizontal axis wind turbine system	
Horizontal axis wind turbine	500 W, 24 V, 18 A
Charge Controller	24 V
Other equipment	
Battery	100 ah, 12 V
Inverter	1200 W, 24 V
AC led floodlights (Resistance, load)	150 W
Data Loggers	
Measurement system	

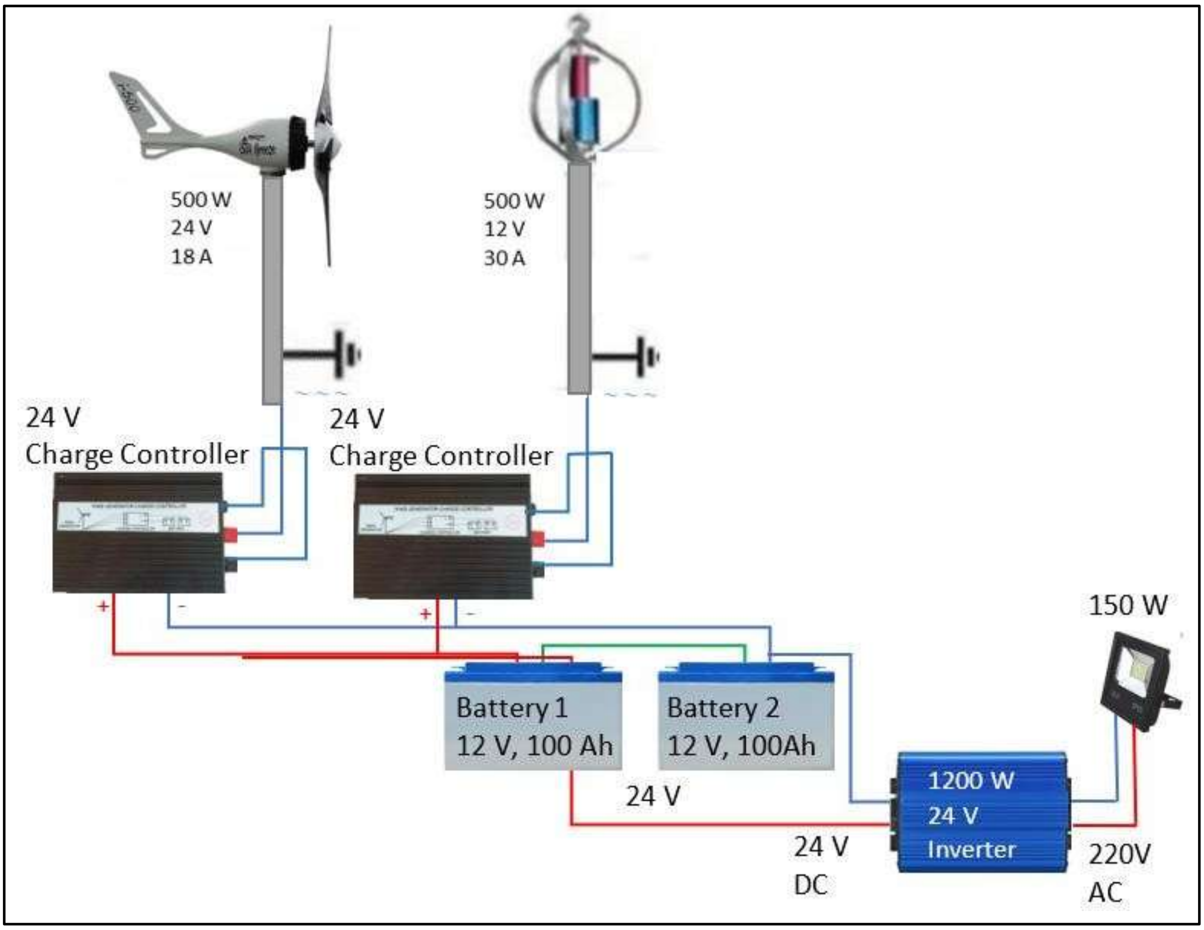


Figure 2. Schematic drawing of the wind energy system

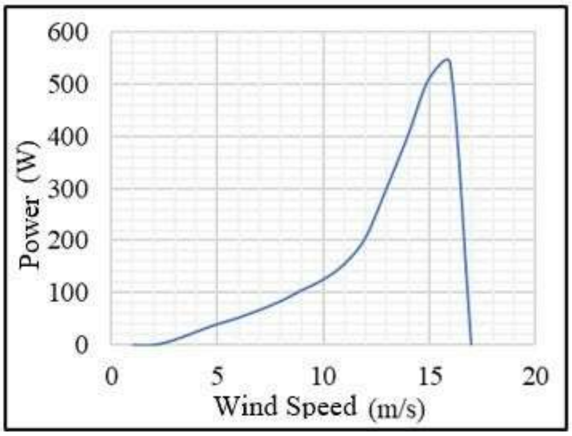
a) b)

Figure 3. a) Horizontal axis wind turbine, b) Vertical axis wind turbine

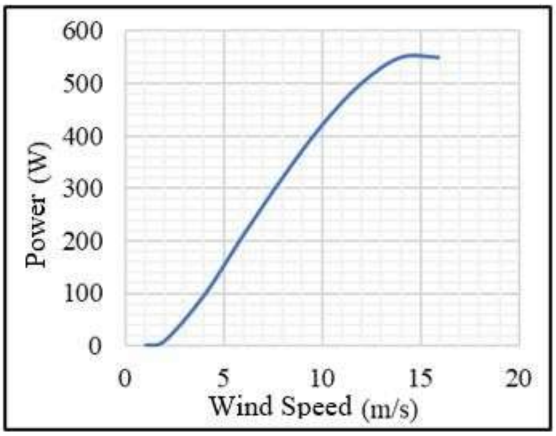
The vertical turbine system consists of two different structures with airfoil and trailing blades. At low wind speeds, the drag blade structure produces energy, while the airfoil blade continues production at high wind speeds. The horizontal axis wind turbine has 3 blades and a rotor diameter of 105 cm and starts producing energy from 3 m/s. Both turbines are installed at the same height and do not interrupt each other's air flow. The technical specifications of the HAWT and VAWT are given in Table 2 and the power curves are shown in Figure 4.

Table 2. Horizontal axis and vertical axis wind turbine technical specifications

Specification	**Horizontal Axis Wind Turbine (HAWT)**	**Vertical Axis Wind Turbine (VAWT)**
Turbine type	 Horizontal axis wind turbine (Three blades)	 Savonius-Darrieus
Rated Power	500 W	500 W
Body material	Composite	Aluminium
Alternator type	Mono-phase	Mono-phase
Alternator material	Aluminium	Aluminium
Starting wind speed	2,0 m/s	1,5 m/s
Working wind speed	2.0 – 15 m/s	1,5 – 15 m/s
Blade number	3	3
Rotation (rotor) diameter	1,25 m	1,1 m
Weight	27 kg	35kg
Blade material	Composite (Carbon)	Aluminium
Output voltage	DC 24 V	DC 24 V
Noise rating	40 dB	35 d
Tower height	3 m	3 m



a)

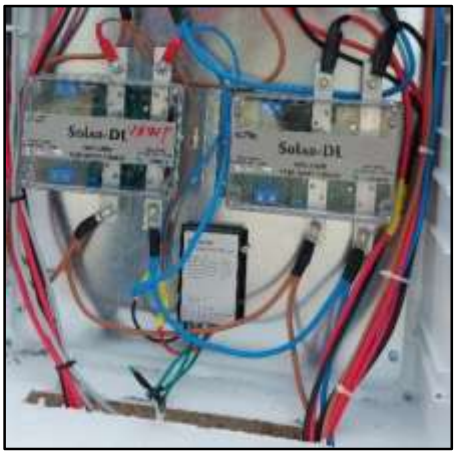


b)

Figure 4. a) Horizontal axis wind turbine power curve and b) Vertical axis wind turbine power curve

Both turbines are installed in such a way that they do not interrupt each other's air flow. Both turbines will be activated at the same time and their performance will be compared under the same climatic and operating conditions. This will determine which type of turbine is more effective for small-scale energy production in Edirne's climatic conditions, for example to meet domestic energy needs, under real field conditions. With the start of field measurements, both theoretical performance calculations and actual performances were measured, and the real efficiencies and capacity factors of each system were determined. Figure 5 shows the off-grid inverter, charge controller and data logger.

a)



b)

Figure 5. a) Off-grid inverter and charge controller, b) Data logger

Meteorological station data, solar radiation, outdoor temperature, wind speed and direction, outdoor humidity measurements, energy production of vertical axis turbines and horizontal axis turbines were simultaneously measured and recorded.

RESULTS AND DISCUSSION

The energy production of HAWT and VAWT systems was measured and recorded simultaneously. The energy production, power profiles and system performances of HAWT and VAWT systems were determined, and their performances were compared by using the data measured under the same climatic and operating conditions with the systems operating simultaneously.

On 10 December 2020, the maximum and average wind speed are shown in Figure 6, the current and voltage values of the HAWT and VAWT systems are shown in Figure 7, and the power generation is shown in Figure 8.

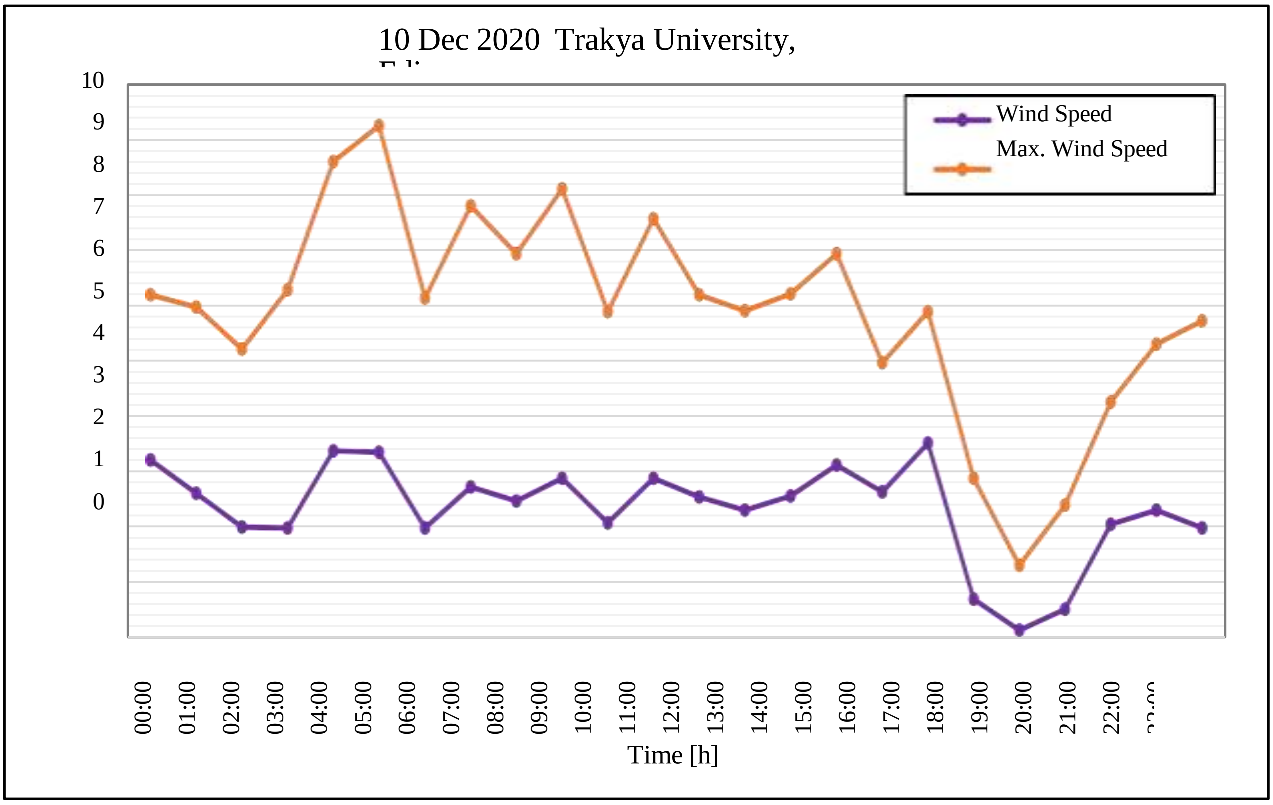


Figure 6. Maximum and average wind speed on 10 December 2020

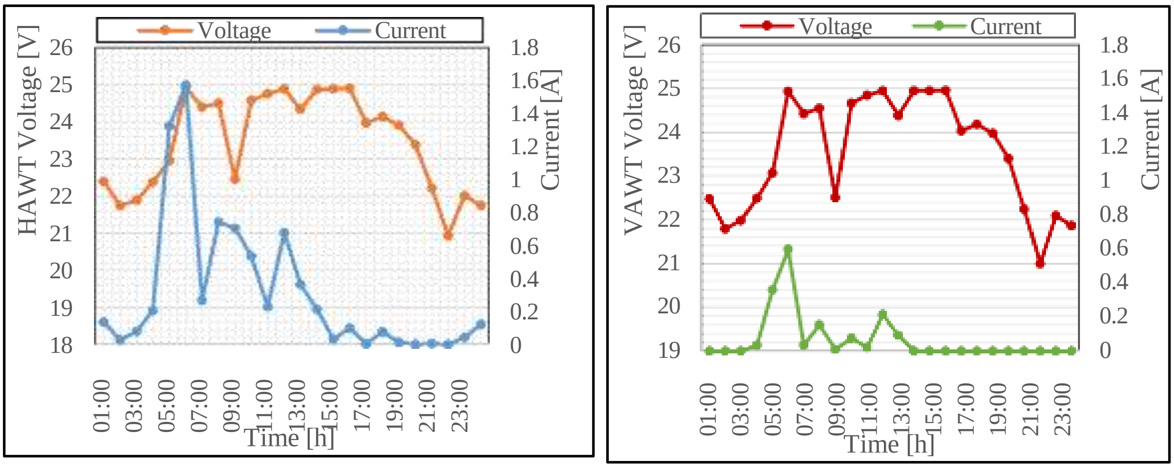


Figure 7. Current and voltage values of HAVT and VAWT systems on 10 December 2020

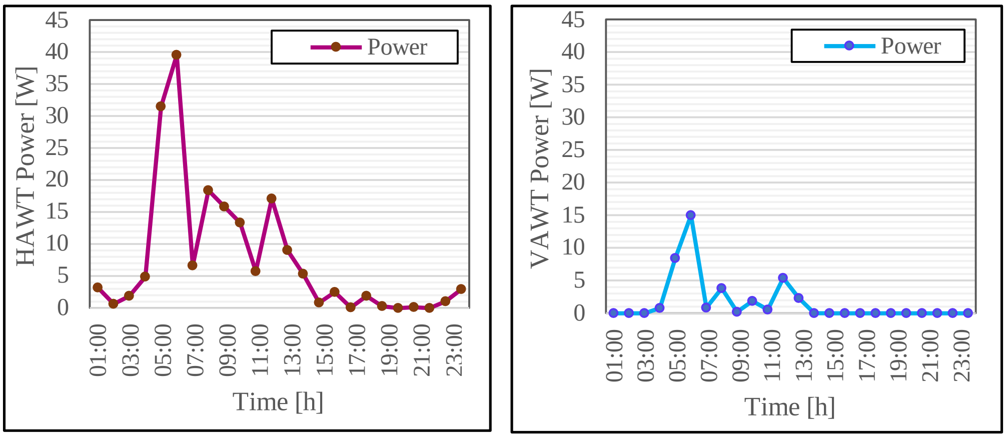


Figure 8. HAVT and VAWT systems power generation on 10 December 2020

In December 2020, the electricity production of the HAWT system and the average daily wind speeds are shown in Figure 9, the electricity production of the VAWT system and the average daily wind speeds are shown in Figure 10, and the comparison of the daily energy production of the HAWT and VAWT systems in December is shown in Figure 11.,

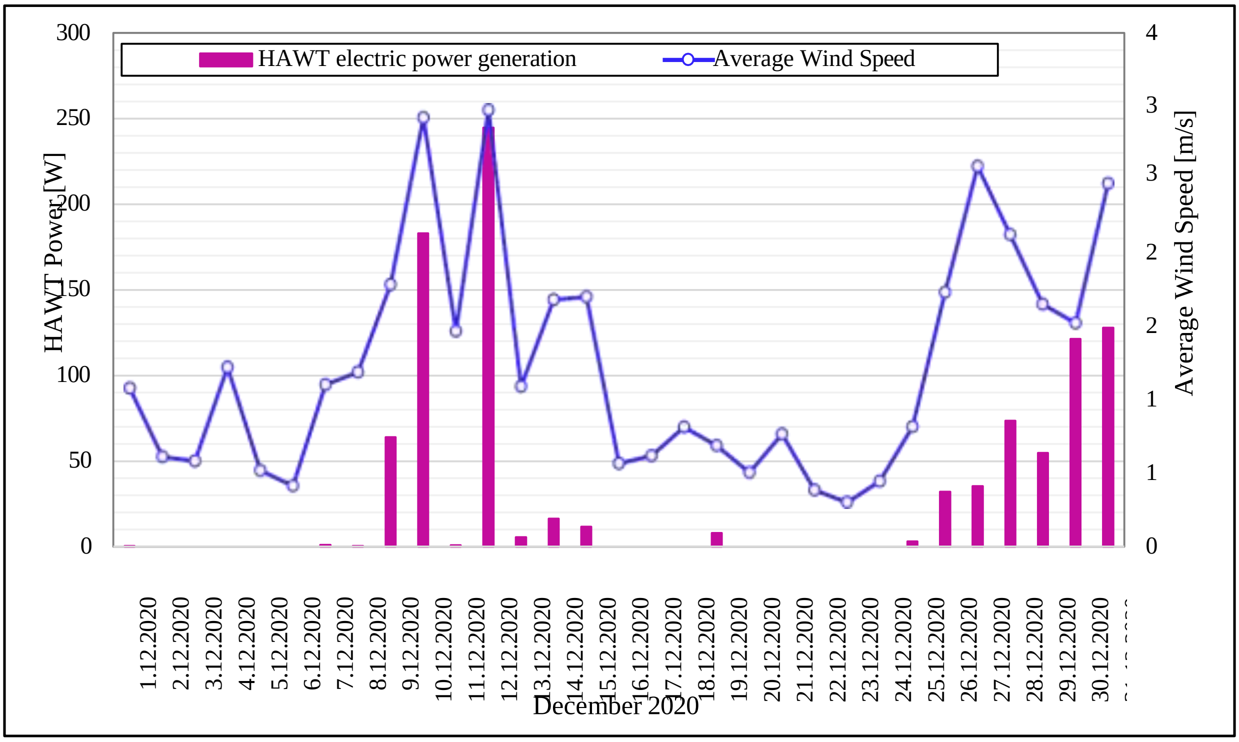


Figure 9. HAWT system electricity generation and daily average wind speed in December 2020

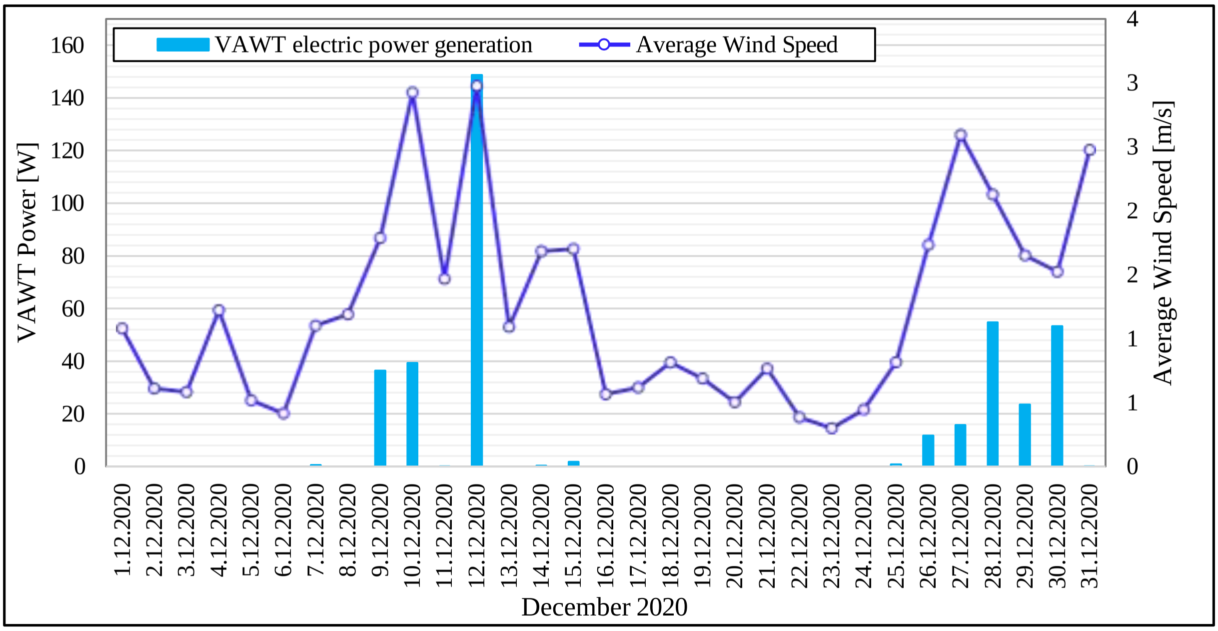


Figure 10. In December 2020, VAWT system power generation and daily average wind speeds

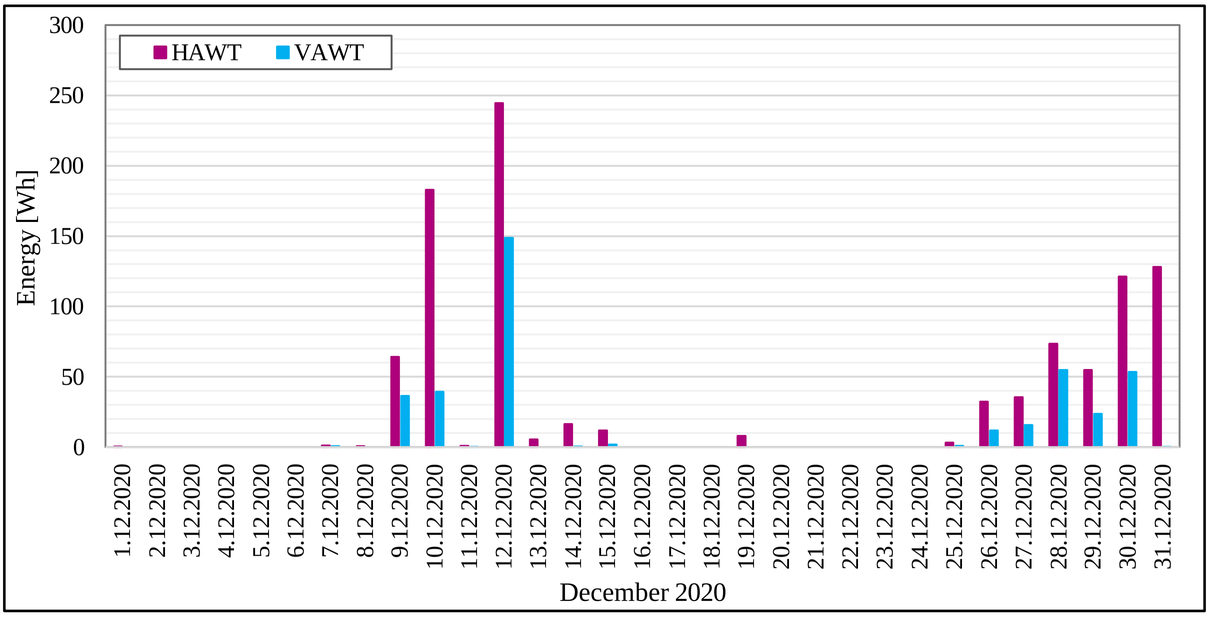


Figure 11. Comparison of energy production of HAWT and VAWT systems daily in December 2020

On 8 January 2021, the maximum and average wind speeds are shown in Figure 12, the current and voltage values of the HAWT and VAWT systems are shown in Figure 13, and the power generation is shown in Figure 14.

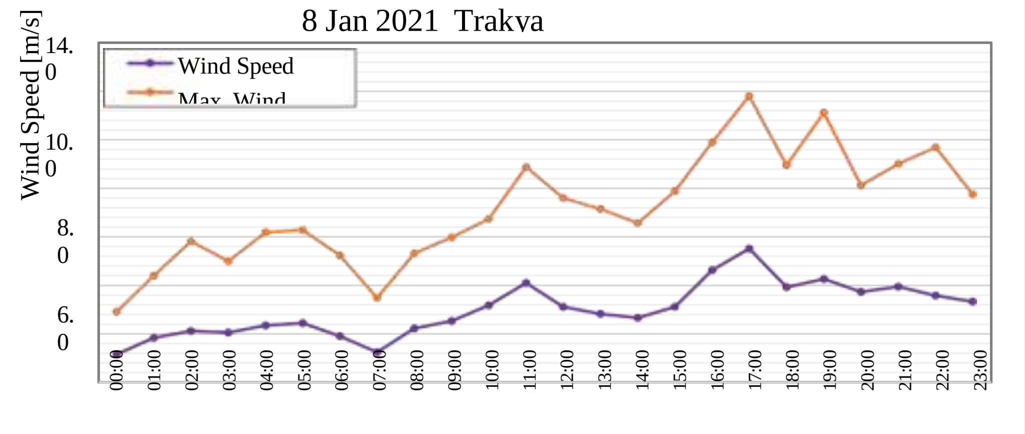
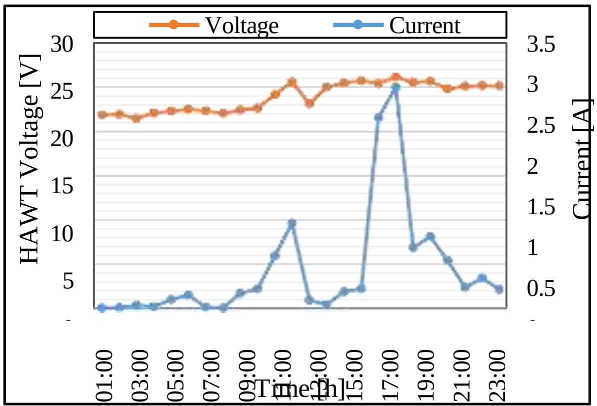


Figure 12. Maximum and average wind speed on 8 January 2021



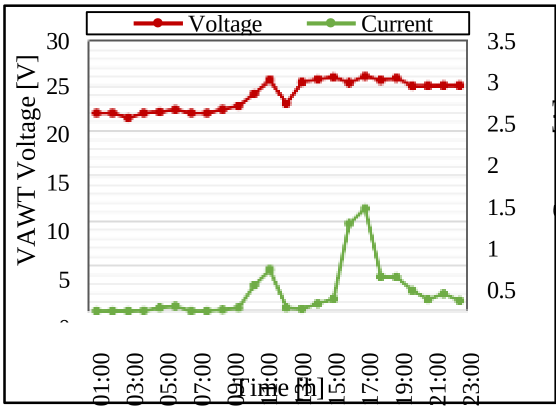
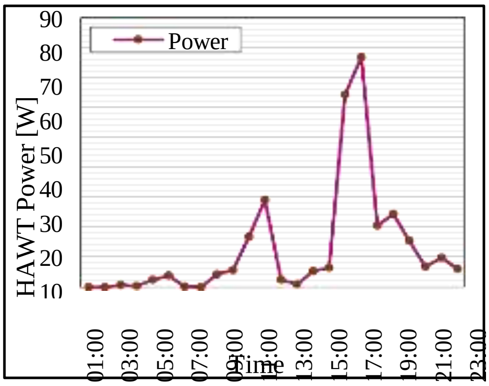


Figure 13. Current and voltage values of HAVT and VAWT systems on 8 January 2021



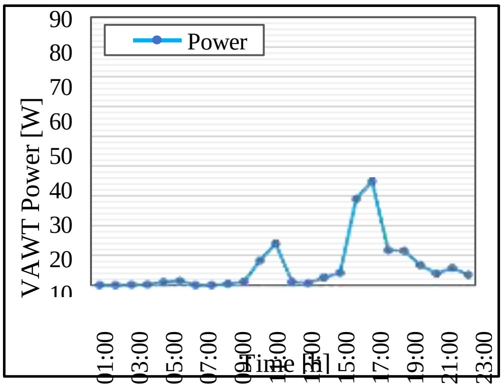


Figure 14. HAVT and VAWT systems power generation on 8 January 2021

In January 2021, HAWT system electricity generation and daily average wind speeds are shown in Figure 15, VAWT system electricity generation and daily average wind speeds are shown in Figure 16, and the comparison of HAWT and VAWT system daily energy generation in December is shown in Figure 17.

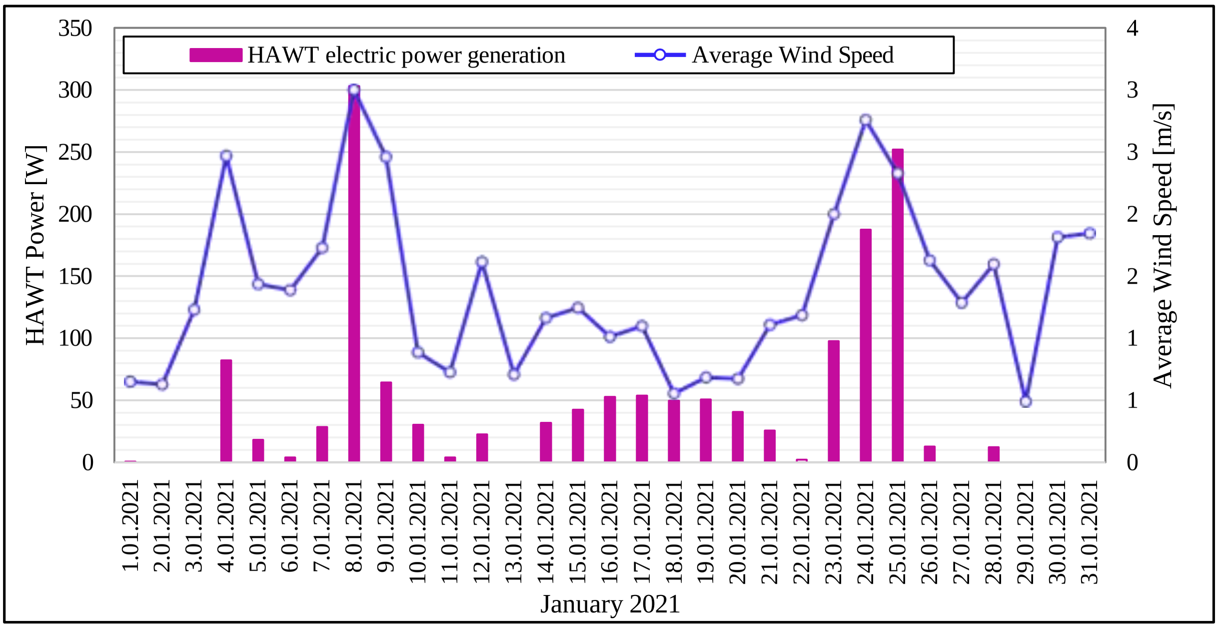


Figure 15. HAWT power generation and average daily wind speed in January 2021

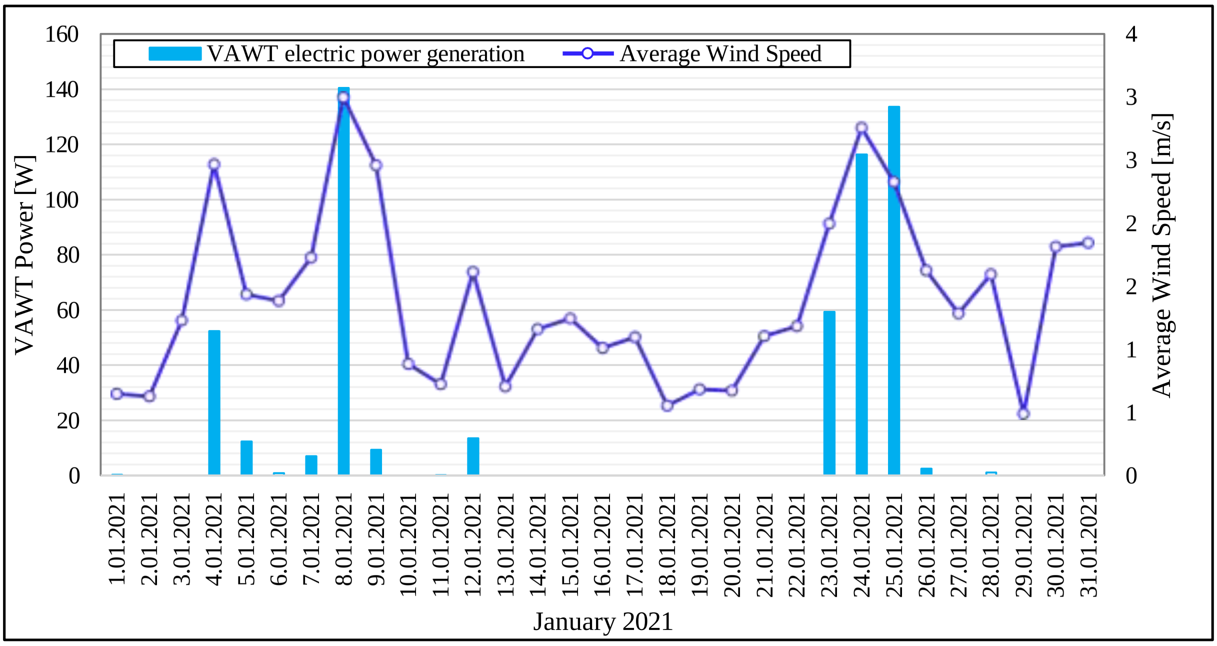


Figure 16. In January 2020, VAWT system electricity generation and average daily wind speeds

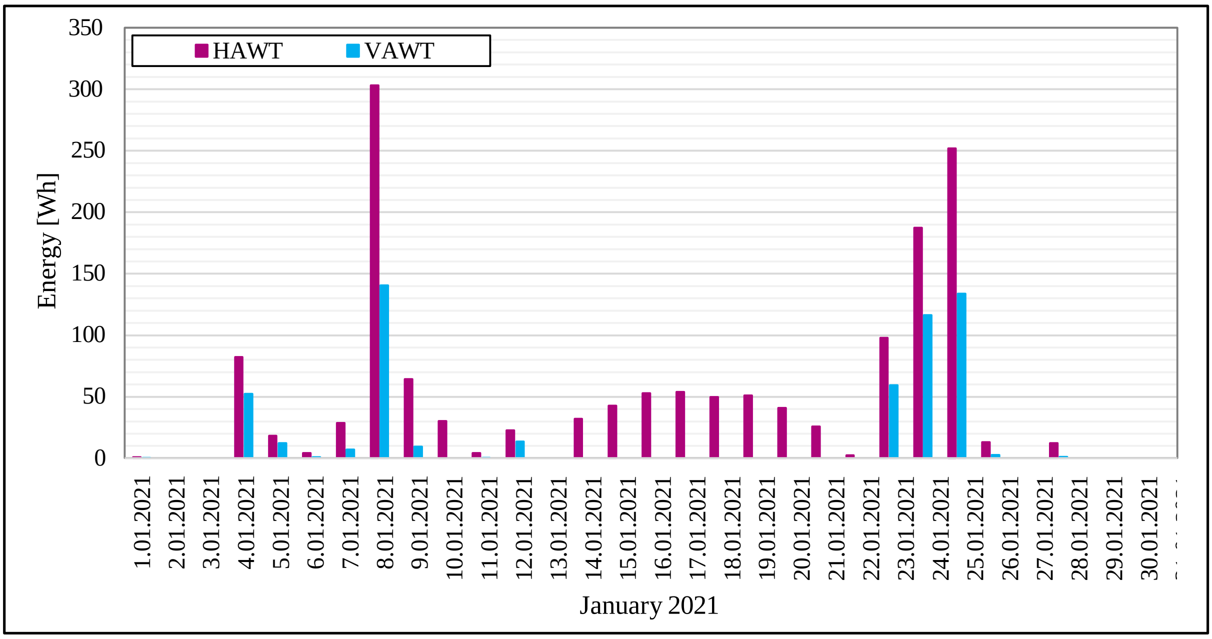


Figure 17. Daily energy production from HAWT and VAWT systems in February 2021

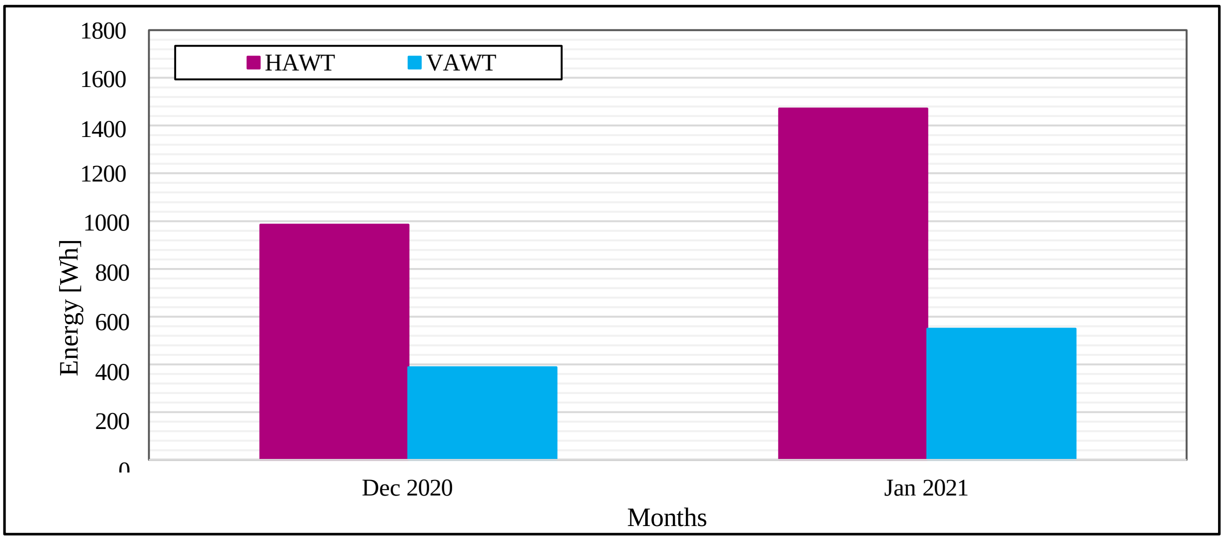


Figure 18. Total energy generated by HAWT and VAWT systems in December 2020 and January 2021

Figure 18 shows the total energy produced by the HAWT and VAWT systems in December 2020 and January 2021. When analysing the electricity generation performance of both systems, the total energy produced by the HAWT system is

984.9 Wh and 1,471.1 Wh, and the total energy produced by the VAWT system is

387.8 Wh and 548.8 Wh in December 2020 and January 2021, respectively. Both systems generated more energy in January. Comparing the energy production in January and December, the HAWT system produced 49.4% more electrical energy and the VAWT system produced 41.5% more electrical energy in January. In addition, the HAWT system produced 153% more electricity in December 2020 and 168% more electricity in January 2021 than the VAWT system.

CONCLUSIONS

In the analyses carried out within the project, the total two-month average electrical energy production of small-scale production applications in Edirne province was determined to be 1,228.0 Wh for horizontal axis wind turbines and 468.4 Wh for vertical axis wind turbines. Based on the results of the analysis, PV/T systems are more suitable for meeting the electricity needs of buildings in Edirne and similar climatic conditions. According to the results of the analysis, the HAWT system provided on average 2.6 times more energy production than the VAWT system.

ACKNOWLEDGEMENT

This study was funded by Trakya University Scientific Research Projects Unit (TÜBAP) (Project No: 2020/31).

REFERENCES

Al-Rawajfeh, M. A., Goma, M. R. 2023. Comparison between horizontal and vertical axis wind turbine. International Journal of Applied Power Engineering (IJAPE). 12(1): 13-23.

Borg, M., Shires, A., Collu, M. 2014. Offshore floating vertical axis wind turbines, Dynamics modelling state of the art. Part I: Aerodynamics. Renew Sustain Energy Rev. 39: 1214-1225.

Eftekhari, H., Al-Obaidi, A. S. M., Eftekhari, S. 2022. Aerodynamic performance of vertical and horizontal axis wind turbines: A comparison review. Indonesian Journal of Science and Technology. 7(1): 65-88.

Eltayesh, A., Castellani, F., Burlando, M., Hanna, M.B., Huzayyin, A.S., El-Batsh, H.M., Becchetti, M. 2021. Experimental and numerical investigation of the effect of blade number on the aerodynamic performance of a small-scale horizontal axis wind turbine. Alexandria Engineering Journal. 60: 3931–3944.

Fadil, J., Ashari, M. 2017. Performance comparison of vertical axis and horizontal axis wind turbines to get optimum power output. In 2017 15th international conference on quality in research (QiR): International Symposium on Electrical and Computer Engineering. 429-433.

Johari, M. K., Jalil, M., Shariff, M. F. M. 2018. Comparison of horizontal axis wind turbine (HAWT) and vertical axis wind turbine (VAWT). International Journal of Engineering and Technology. 7(4.13): 74-80.

Kouloumpis, V., Sobolewski, R.A., Yan, X. 2020. Performance and life cycle assessment of a small scale vertical axis wind turbine. Journal of Cleaner Production. 247-119520: 1-11.

Paraschivoiu, I., Ammar, S., Saeed, F. 2018. VAWT versus HAWT: a comparative performance study of 2–6 MW rated capacity turbines. Transactions of the Canadian Society for Mechanical Engineering. 42(4): 393-403.

Peng, H.Y., Liu, H.J., Yang, J.H. 2021. A review on the wake aerodynamics of H-rotor vertical axis wind turbines. Energy. 232, 121003: 1-23.

Pourrajabian, A., Dehghan, M., Rahgozar, S. 2021. Genetic algorithms for the design and optimization of horizontal axis wind turbine (HAWT) blades: A continuous approach or a binary one. Sustainable Energy Technologies and Assessments. 44: 101022.

Simmons, D.M. 1975. Wind Power, Noyes Data Corporation, Park Ridge, California.

Thrace Development Agency. 2012. TR21 Thrace Region Energy Report. 466 https://www.trakyaka.org.tr/upload/Node/33092/xfiles/enerji_raporu.pdf.

Zhao, P., Jiang, Y., Liu, S., Stoesser, T., Zou, Li., Wang, K. 2006. Investigation of fundamental mechanism leading to the performance improvement of vertical axis wind turbines by deflector. Energy Conversion and Management. 247-114680: 1-15.

Zhao, Z., Wang, D., Wang, T., Shen, W., Liu, H., Chen, M. 2022. A review: Approaches for aerodynamic performance improvement of lift-type vertical axis wind turbine. Sustainable Energy Technologies and Assessments. 49, 101789: 1-20.

https://enerji.gov.tr/bilgi-merkezi-enerji-ruzgar
https://repa.enerji.gov.tr/REPA/iller/EDIRNE-REPA.pdf

REPRODUCTIVE STATUS OF BLUE SWIMMING CRAB (*PORTUNUS PELAGICUS*) AND INFLUENCINGENVIRONMENTAL FACTORS IN PARE-PARE BAY, SOUTH SULAWESI, INDONESIA

Yushinta Fujaya[1]*, Andi Aliah Hidayani[1], Moh. Tauhid Umar[1], Matthew B Ogburn[2*], Robert Aguilar[2], Henry D Legett[2]

[1]Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar 90245, Indonesia;

[2]Smithsonian Environmental Research Center, Edgewater, MD 21037, USA

*Email: yushinta.fujaya@unhas.ac.id; OgburnM@si.edu

ABSTRACT

Background. Blue swimming crab (*Portunus pelagicus*) is an important fishery in the Indo-Pacificregion, including Indonesia. However, many *P. pelagicus* populations are experiencing over- exploitation. Maintaining healthy reproductive output and potential recruitment is a crucial step inconserving and managing crab stocks. **Aims**. We assessed spawning potential of *P. pelagicus* by examining sex ratio, spawning season, size of ovigerous females, fecundity, and environmental factors. **Methods**. We sampled crabs captured in a small-scale local fishery in Pare-Pare Bay, South Sulawesi, Indonesia. A total of 5278 crabs were collected from monthly samples collected July 2023 through June 2024. We used Chi-square (χ^2) tests to compare sex ratios. A principal component analysis (PCA) was used to determine the influence of environmental factors on the abundance of ovigerous females. **Results**. Ovigerous crabs were most abundant in the 8-11 cm size range. The average sex ratio was about 0,6:0,4 (M:F). The proportion of female catch that wasovigerous ranged from 11% in May to 32% in March. The fecundity ranged from 107,640 eggs to1,308,400. The environment condition at that time is Salinity (32,2 □ 1,7 ppt), Dissolve Oxygen (7,2 □ 0,8 ppm),pH (8,3 □ 0,0), temperature (33,5 □ 0,3 ^{o}C), and current (0,063 □ 0,009 m/s). **Conclusion**. Sex ratio and the abundance of ovigerous female crabs fluctuates throughout the yearwithin the small-scale fishery. 34 % of the ovigerous females are under the legal size limit (< 10 cm). While crabs in Pare-Pare Bay spawn throughout the year, we observed peaks in activity, withthe highest in March. Water temperature and pH had the strongest association with the abundanceovigerous crabs. It shows that there is a strong influence between spawning season and environment, especially the temperature. The results of this research indicate that the potential forspawning blue swimming crabs in Pare-Pare Bay is still good, but great attention is needed to limitthe capture of female crabs, especially those that ovigerous female for sustainability and protect the habitat damage.

Keywords: Crab, Environment, Pare-Pare Bay, *Portunus pelagicus*, Reproduction

INTRODUCTION

Blue swimming crab (*Portunus pelagicus*) is an important fishery commodity worldwide, including Indonesia. However, the threat of over-exploitation is urgent and widespread, with decreased catches and increasingly small sizes becoming prevalent issues. Hamid et al. (2016) reported that blue swimming crab stocks in Lasongko Bay, Central Buton, Indonesia, have been overfished and are considered critical. Thus, effective management is necessary. La Sara et al. (2019) also reported that in Southeast Sulawesi waters, fishing activities of blue swimming crabs had reached an over-exploitation level. Ernaningsih et al. (2024) reported that the exploitation rate of blue swimming crabs from the Bawasalo Waters Pangkep Regency, South Sulawesi, is about E

= 0.55. It means that the stock of *P. pelagicus* was slightly higher than that of an optimally exploited stock. Nurdin et al. (2022), from the research in Spermonde Archipelago, Indonesia, stated the blue swimming crab stock status in Spermonde is over-fishing with an SPR value of 7%. However, Tirtadanu and Chodrijah (2019) reported that the exploitation status of blue swimming crabs in Kwandang Waters, North Sulawesi, Indonesia, showed no overfishing, based on the current fishing mortality (Fcur=0,86), which was less than the reference point F0.1=2,8 and based on the estimated SPR of 24% so the fishing effort could continue. The government could implement the minimum legal size of 117 mm CW for the future management strategy.

One crucial step to save stock is to maintain healthy reproductive activity. Reproductive status was used as an indicator to identify spawning habitat, and data from long-term fishery- independent surveys were used to evaluate spatiotemporal trends in the abundance of spawning females (Ogburn and Habegger, 2015) and to assess the sustainability of crab fishery. According to Hilborn 2004 and Purcell, 2004 in Aguilar et al. (2018), successful rebuilding of depleted stocks through stock enhancement, a process of increasing the abundance of a fish stock through human intervention, can occur only when the factors responsible for the stock decline are addressed concurrently through sound fishery management.

Spawning season and fecundity are essential information for understanding the complete status of crab populations. Basic information regarding the reproductive biology of P. pelagicus can help policymakers and managers establish regulatory measures to conserve populations. More than that, studying reproductive biology, especially spawning season, concerning environmental conditions such as Salinity, Temperature, and pH is essential information for developing crab hatchery technology to support cultivation.

In this paper, the sex ratio of males and females, spawning season, size of females at sexual maturity, size at 50% of ovigerous females, fecundity, and environmental factors that affect the spawning season are presented for P. pelagicus in Pare-Pare Bay. Understanding the reproductivebiology of P. pelagicus is essential for sound scientific advice for future fisheries management. It opens up possibilities for stock enhancement and hatcheries, providing a hopeful outlook for the future of crab populations.

MATERIAL AND METHODS

The research area, Pare-Pare Bay, South Sulawesi, Indonesia, is of significant importance due to its unique characteristics and its role as a habitat for P. pelagicus. The bay, with latitudes $119^o34,440$'E to $119^o38,220$'E and $4^o1,660$'S to $3^o58,140$'S, is a bay on the western coast of South Sulawesi Province that faces the Makassar Strait. Crabs were sampled at the crab landing site in Watang Suppa Village, Suppa District, Pinrang Regency (Figure 1). The crab samples were caught by fishermen using gill nets with a length of 21 m, a height of 0.46 m, and a mesh size of 3 inches. Were 5278 samples collected from July 2023 to June 2024 at the landing site. The crab data was measured in situ, and fecundity was measured at the Hatchery Laboratory, Faculty of Marine and Fisheries Sciences, Hasanuddin University. Environmental parameters were taken from four stations, which were determined based on differences in bottom substrate and vegetation.

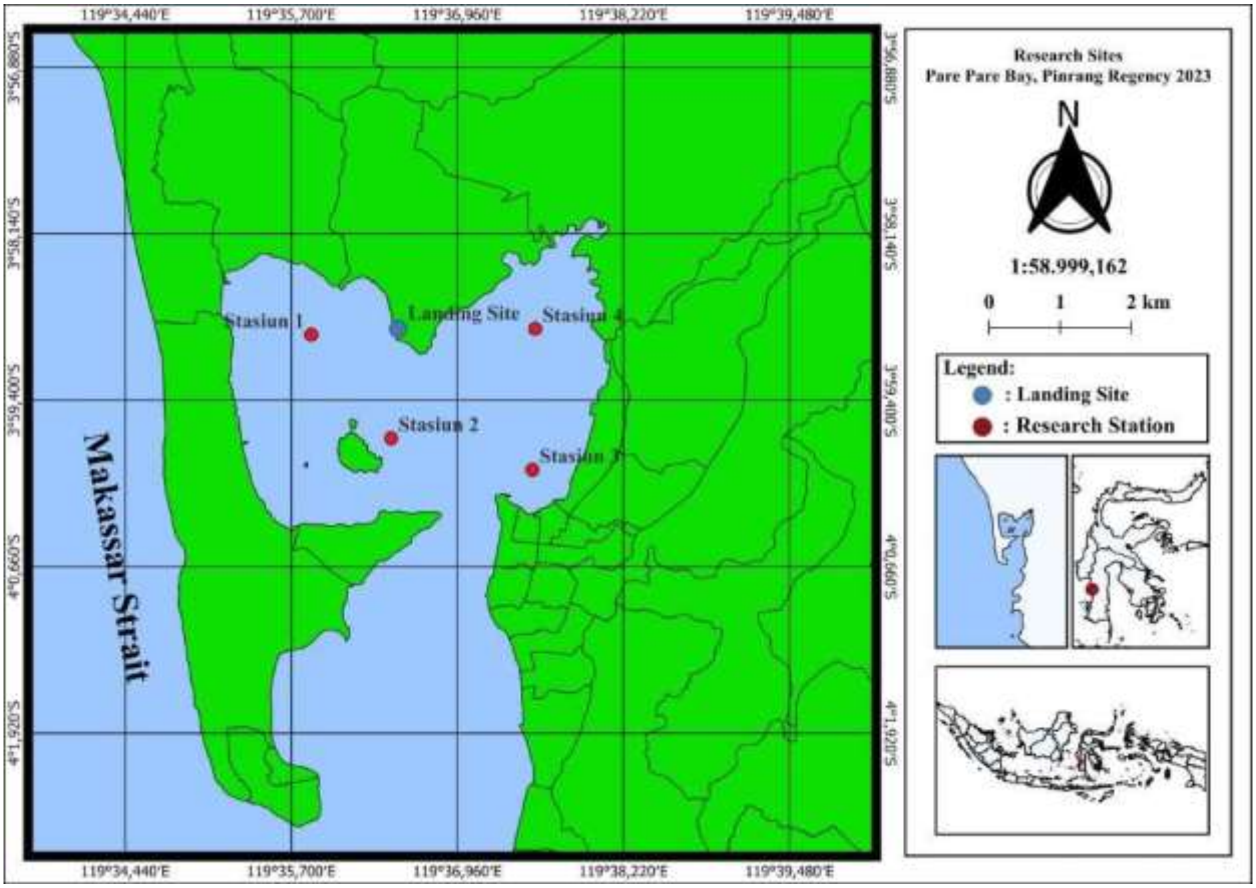


Figure 1. Locations of sampling sites for *Portunus pelagicus* in the Pare-pare Bay South Sulawesi,Indonesia.

Crab Size

The crab sizes measured were outer carapace width, body weight, and egg mass weight. Outer carapace width is the distance between the most extended spine on the right and left side ofthe crab's body. Carapace width was measured using a caliper with an accuracy of 0.01 mm, and weight was measured using a digital scale with an accuracy of 0.01 g. The first step is male, female, and ovigerous, differentiated crabs. Then, the size and condition of the crabs were recorded. Onlyintact crabs were used to analyze length-weight relationships and size comparisons between sexes.The egg mass (sponge) is taken out from under the abdomen and weighed together with the pleopod where it is attached.

Sex-Ratio

In this research, sex ratio analysis was carried out by monthly data sets of the total number of male and female crabs (Figure 2). Gender can be identified based on the shape of the abdomen. Male crabs have a more tapered abdomen, while females have a broader shape. Apart from being based on the shape of the abdomen, gender can also be determined from the color of the crab itself. The bright blue color with white spots on the shell indicates the male, while the darker color indicates the female crab, as in Figure 2.

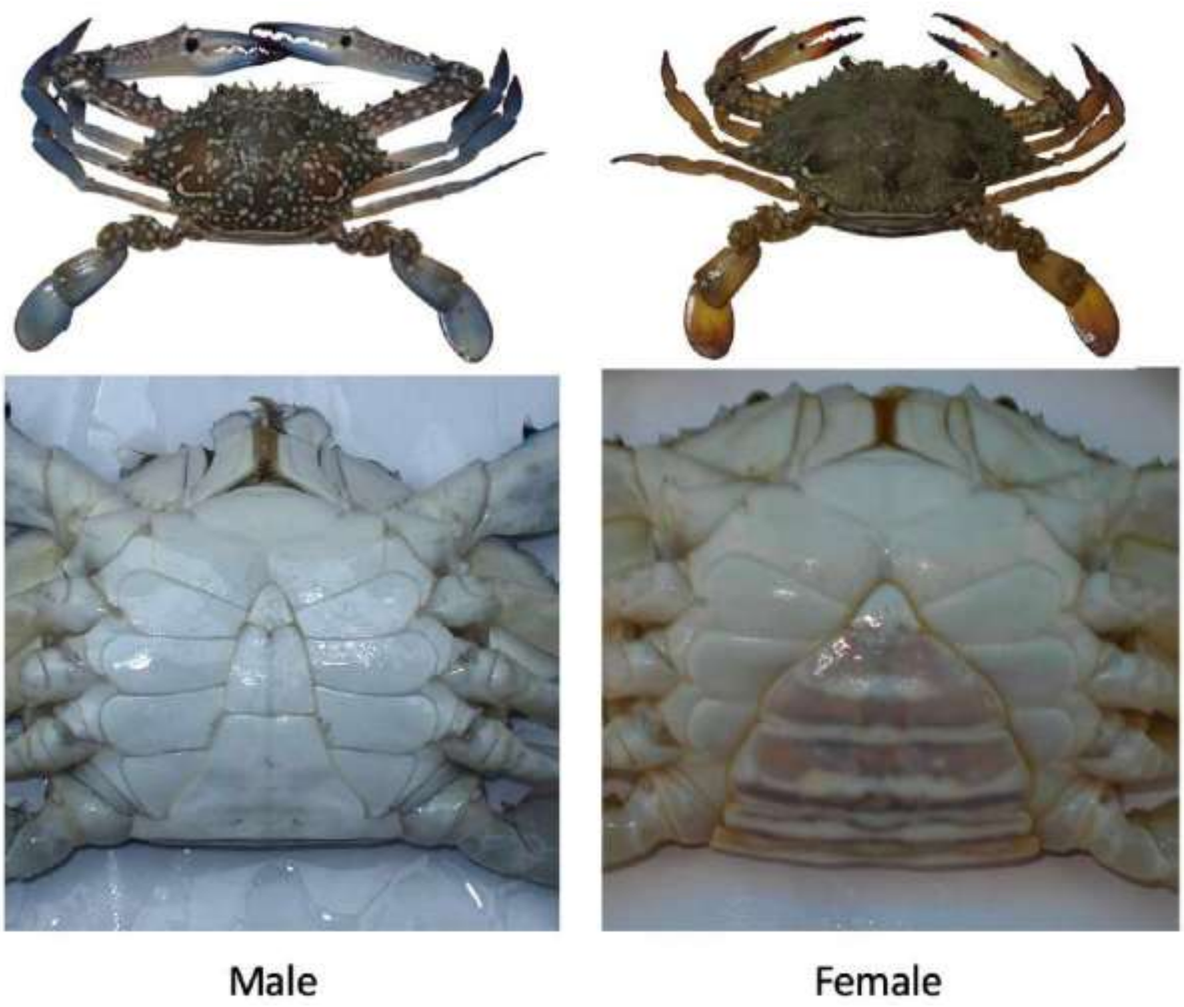


Figure 2. Male and female Blue Swimming Crab (Portunus pelagicus)

Spawning Season

The spawning season of this species was determined by the percentage of ovigerous females each month. An ovigerous female is a crab carrying eggs under the abdomen or apron (Figure 3). The ovigerous female used in this study is just a crab carrying eggs in orange color.

Figure 3. The ovigerous female

Fecundity

Fecundity is calculated as the number of eggs carried externally by the female (Kumar et al., 2000). This study sampled 129 ovigerous females from crab landing sites with a wide range ofsizes. Caliper measured the carapace width of each ovigerous female to the nearest 0,01 mm. Egg-bearing pleopods were removed carefully; the wet weight of the whole egg mass was measured tothe nearest 0.01 g using an electronic balance. The corresponding carapace width to the egg sac mass was also recorded. Approximately 0.1 g sub-samples were taken from three different placesof the egg sac. Egg samples were preserved with Gilson Solution until eggs were dispersed and were ready to count. Gilson's solution: 100 mL 60% ethanol or methanol, 880 mL water, 15 mL 80% nitric acid, 18 mL glacial acetic acid, and 20 g mercuric chloride (Snyder, 1983 in Lowerre-Barbieri and Barbieri, 1993). The total fecundity was estimated using the following equation:

Where w = the number of eggs in the sub-sample, W = the total weight of the sub-sample, and OW

$$TF = \frac{w}{W} * OW$$

= the total weight of the preserved ovaries of the particular specimen. Relative fecundity was calculated as the number of eggs per gram of body weight. Using an Excel program, regression analyses were performed to establish the relationships between fecundity (F) and carapace width (CW).

Environmental parameters measured

The environmental parameters measured in this study were Dissolved oxygen (DO), Salinity, pH, Temperature, water depth, and current speed. Measure DO in water using a DO meter, Salinity using a hand refractometer, pH using a pH meter, Temperature using a thermometer, water depth using a Secchi disk, and water current using a current kite. The current kite is put into the water at the same time as activating the stopwatch and then letting the current carry it along a ropeof a predetermined length. When the rope is straight, turn it off, and then note how long it takes toreach the specified distance. All environmental data is taken every month in the morning around 7

- 8 am.

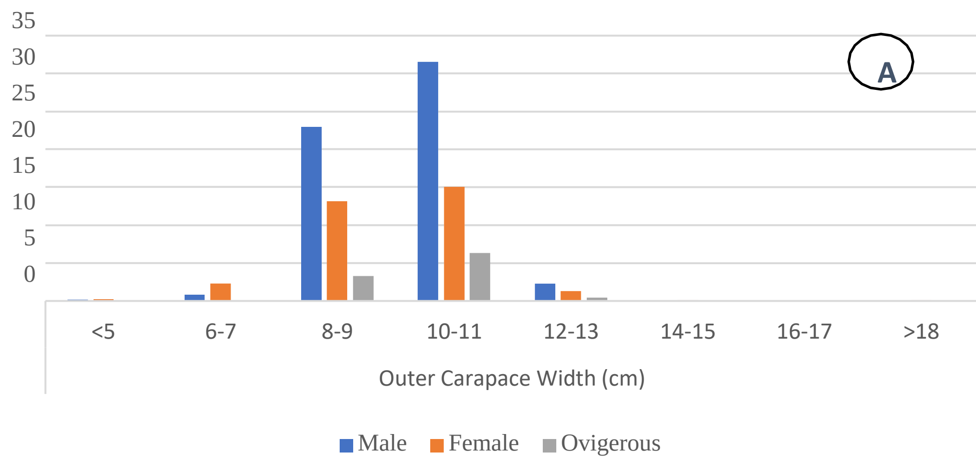
Data Analysis

Chi-square (X^2) statistical was performed to test the difference between ratios in both sexes. To determine the influence of physico-chemical parameters of water on the abundance of ovigerous females in each month, the Principal component analysis (PCA) by Xlstat software wasused (Yao et al., 2012).

RESULT

Crab Sizes

In this research, 3021 male crabs and 2218 females were collected. Among females, there were 1876 non-ovigerous and 342 ovigerous. The size of the crabs at the Watang Suppa village crab landing varies. Both males, females, and those who are ovigerous are dominated by crabs with a Carapace Width of 8-11 cm and weight 31-90 g (Figure 4).



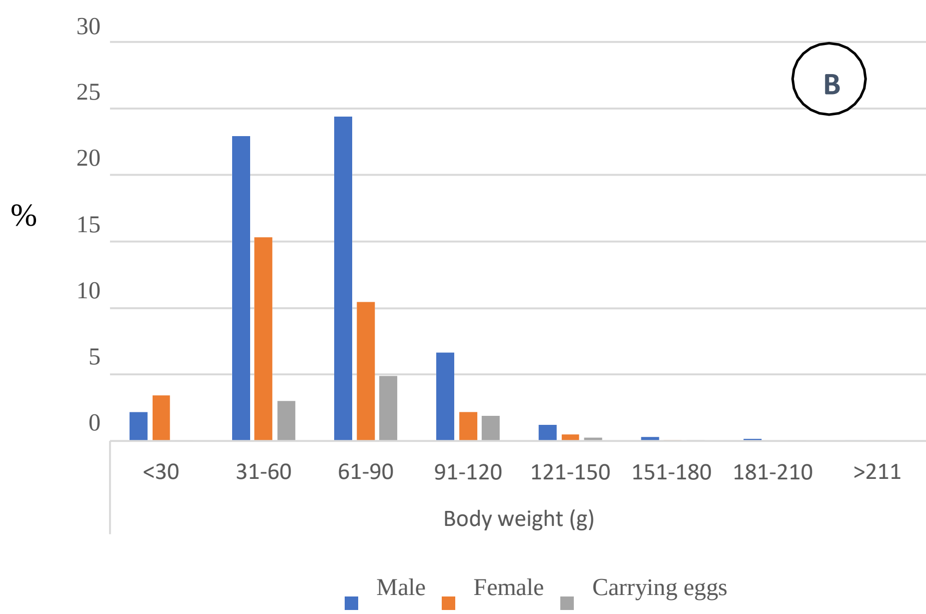


Figure 4. Size distribution of crabs landed from July 2023 to June 2024. A, based on Carapacewidth, and B, based on weight.

Sex Ratio

It was found that the average sex ratio varied every month, and there was a tendency for males to have more numbers than females. The condition of sexual imbalance occurred during observations from January to June 2024 (Figure 5).

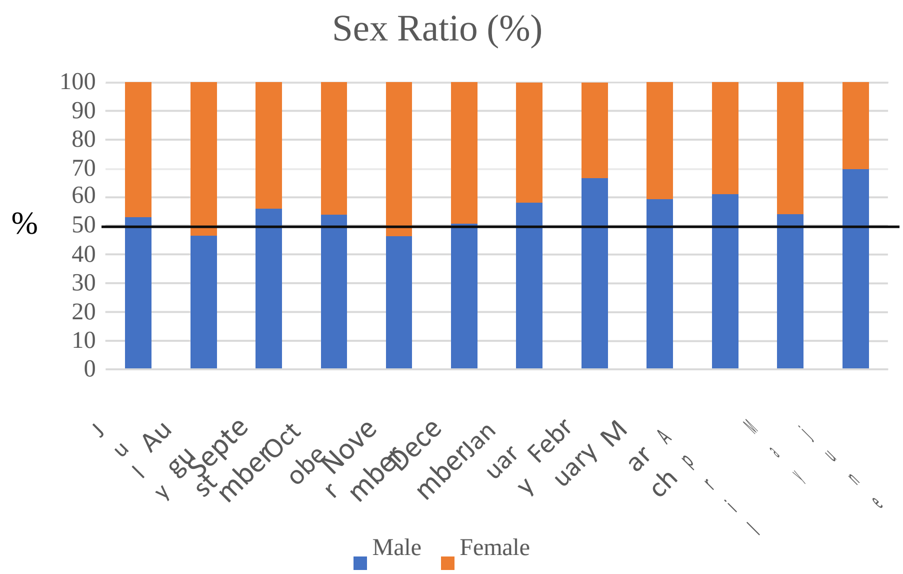


Figure 5. Sex ratio (%) of crabs landed by month (N=5278)

The overall male-female ratio from July 2023 to June 2024 is 1.3: 1, but based on the Chi-square test, the different ratio is non-significant ($p > 0.05$) or statistically, the ratio is comparable to 1:1. However, the Chi-square test results by month is significant ($p<0.05$), this means that thereare disproportionate ratios (not 1:1), namely in February, April, and June 2024, while the other sampling months have comparable ratios.

Spawning Season

Based on observations of ovigerous crabs that were caught and landed, it can be seen that crabs in Pare-Pare Bay spawn throughout the year. However, there are peaks in spawning in certain months, and the highest is in March (Figure 6).

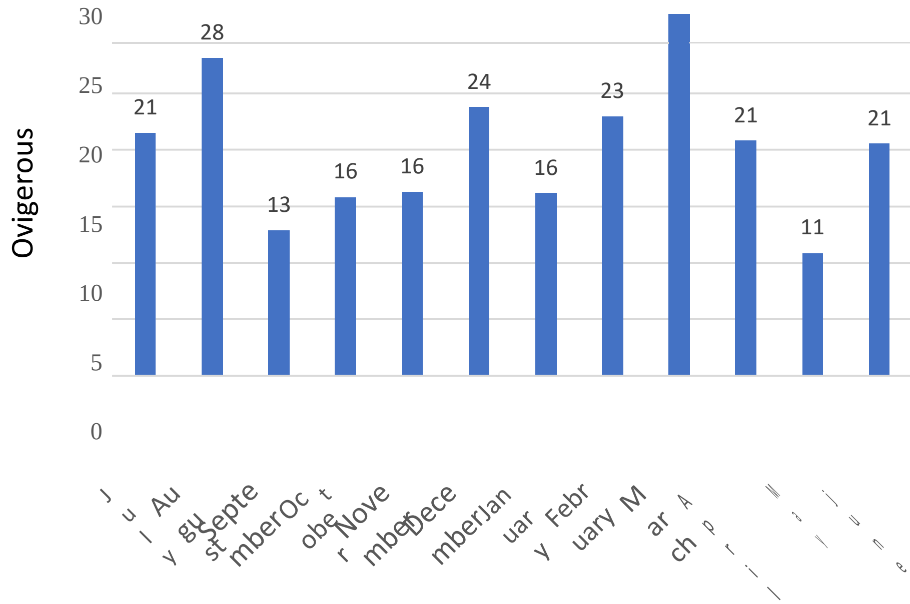


Figure 6. The monthly proportion (%) of ovigerous crabs landed in during July 2023 – June 2024

Fecundity

The fecundity of ovigerous females varied depending on the size and biomass of eggs. In this study, the fecundity of crab eggs found in Pare-Pare Bay ranges between 107,640 eggs for crabs with a carapace width of 8 cm to 1,308,400 for crabs larger than 12 cm. The relationship between carapace width and fecundity follows the equation, $y = 14218e^{0,3289x}$, with the coefficient of correlation R = 0,8230 (Figure 7).

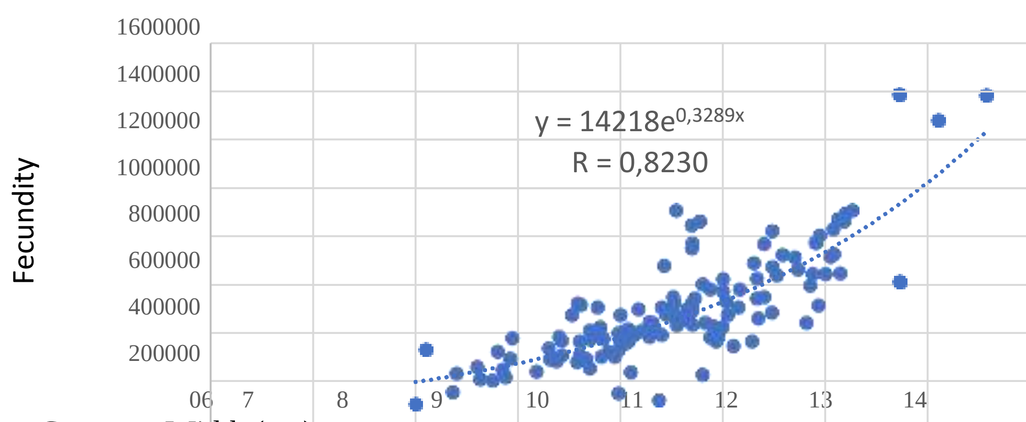


Figure 7. The relationship between carapace width and fecundity

Environmental Parameters

The environmental conditions in Pare-Pare Bay are very dynamic. Water salinity ranges between 30-38 ppt, Dissolved Oxygen 5,4 – 9,2 ppm, pH 7,8 – 8,29, Temperature between 28 – 35, Depth 1,5 – 11,67 m, and current about 0,059 – 0,330 m/s (Table 1).

Table 1. The average value of environmental parameters at each station in Pare-Pare Bay from July2023 to June 2024.

Years	Months	Salinity (ppt)	Dissolve Oxigen (ppm)	pH	Temperature (ºC)	Depth (m)	Current (m/sec)
2023	July	34,3 +/- 1,7	6,6+/- 0,5	7,9 +/- 0,1	28,9 +/- 2,7	5,4 +/- 4,1	0,244 +/- 0,086
	August	34,3 +/-1,6	6,4 +/- 0,4	7,9 +/- 0,1	28,5 +/- 2,6	5,8 +/- 4,2	0,201 +/- 0,070
	September	34,3 +/- 1,6	6,4 +/- 0,4	7,9 +/- 0,1	28,5 +/- 2,6	5,8 +/- 4,2	0,108 +/- 0,091
	October	32, 9 +/- 0,9	7,1 +/- 0,6	8,1 +/- 0,0	30,3 +/- 0,5	5,1 +/- 3,8	0,180 +/- 0,079
	November	33,0 +/- 0,4	6,4 +/- 0,7	8,2 +/- 0,0	31,8 +/- 0,6	4,8 +/- 1,7	
	December	30,8 +/- 1,0	6,2 +/- 0,9	8,1 +/- 0,7	31,3 +/- 1,0	6,0 +/- 4,2	
2024	January	30,5 +/- 0,4	6,3 +/- 0,8	8,2 +/- 0,0	32,8 +/- 1,4	4,8 +/- 3,1	0,079 +/- 0,010
	February	30,4 +/- 3,8	6,0 +/- 1,2	8,2 +/- 0,0	34,8 +/- 0,6	3,5 +/- 1,7	0,087 +/- 0,027
	March	32,2 +/- 1,7	7,2 +/- 0,8	8,3 +/- 0,0	33,5 +/- 0,3	4,8 +/- 3,1	0,063 +/- 0,009
	April	32,3 +/- 1,3	7,7 +/1,0	8,2 +/- 0,0	33,5 +/- 1,0	3,0 +/ 0,8	0,157 +/- 0,095
	May	32,6 +/- 2,1	7,4 +/- 0,1	8,4 +/- 0,1	32,6 +/- 0,5	4,8 +/- 2,1	0,127 +/- 0,024
	June	37,0 +/- 0,8	6,8 +/- 0,5	8,3 +/- 0,0	30,5 +/- 0,6	6,5 +/- 3,3	0,124 +/- 0,030

The results of PCA analysis showed that there was a relationship between spawning seasonand various environmental parameters. Three main factors had Eigenvalues greater than one (>1)with variability respectively 52.4, 21.62, and 16.99 % (Table 2). This shows that factor 1, factor 2, and factor 3 have the most significant influence on crab spawning in Pare-Pare Bay. Factors 1,2, and 3 can be seen from the essential values obtained in factors loading in the supplementary variable: pH and Temperature as factor 1, Salinity and Dissolve Oxygen as factor 2, and current asfactor 3 (Table 3).

Table 2. Eigenvalues of environmental factors in Pare-Pare Bay

	Factor1	Factor2	Factor3	Factor4	Factor5	Factor6
Eigenvalue	3.14	1.30	1.02	0.28	0.24	0.02
Variability (%)	52.40	21.62	16.99	4.64	3.99	0.35
Cumulative %	52.40	74.02	91.01	95.66	99.65	100.00

Table 3. Factor loading of supplementary variables

	Factor1	Factor2	Factor3	Factor4	Factor5	Factor6
Salinity	-0.677	0.520	-0.364	-0.364	0.082	0.017
Dissolve Oxygen	0.285	0.904	0.168	0.138	-0.231	0.023
pH	0.779	0.362	-0.425	0.122	0.249	-0.068
Temperature	0.979	-0.044	0.006	-0.068	0.151	0.111
Depth	-0.754	-0.055	-0.577	0.304	0.012	0.058
Current	-0.687	0.271	0.587	0.123	0.308	0.011
FCE	0.006	-0.100	0.032	-0.079	0.034	0.837

Note: FCE, Female Carrying eggs

Based on a biplot between Factor 1 (F1) and Factor 2 (F2), there is a cumulative variability of 52,40% and 74.02%. The Biplot image shows that four variables have high vector values, namely Temperature, DO, pH, and Salinity, but Temperature has the most significant influence onfemales carrying eggs (FCE) (Figure 8).

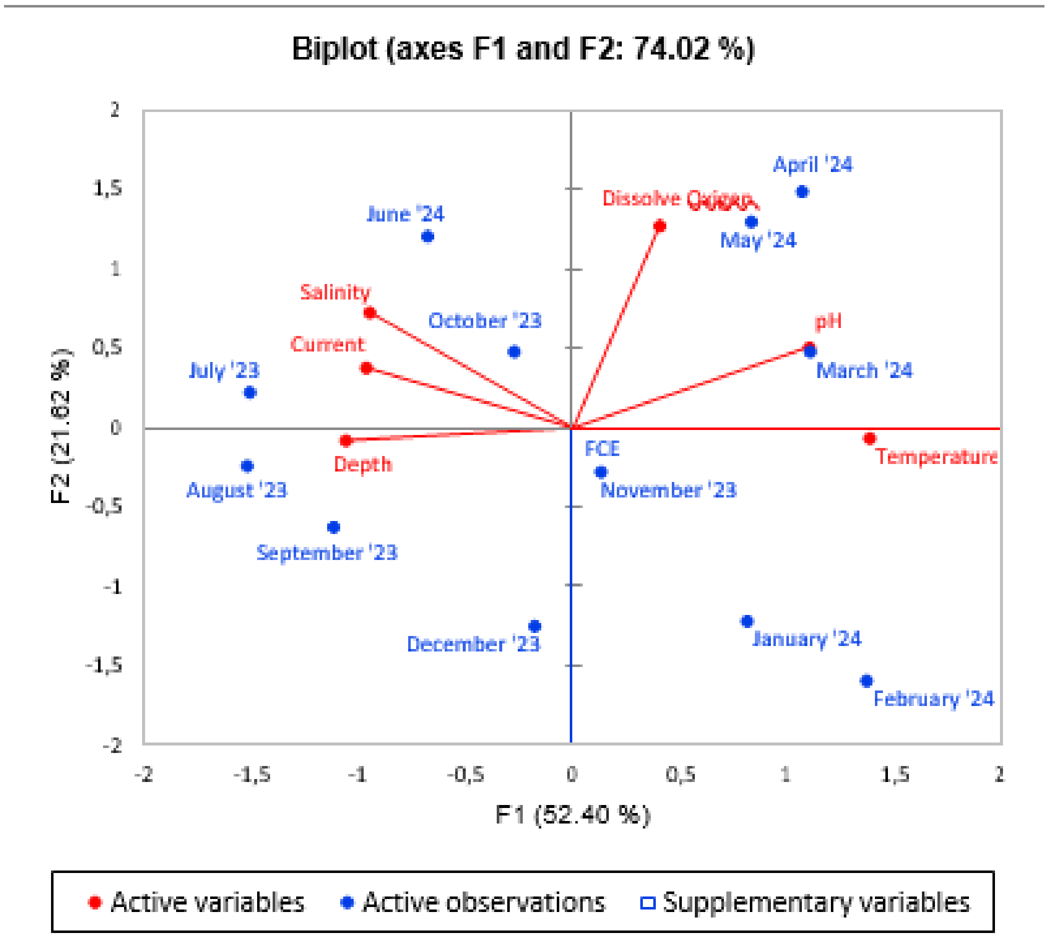


Figure 8. The relationship between physicochemical environmental parameters and the abundance of spawning crab crabs in the waters of Pare-Pare Bay using Principal Component Analysis (PCA)

DISCUSSION

Based on this study, 43% of crab landings are under-sized based on the Indonesian government regularly, and 10% of those crabs are ovigerous (Figure 4). The catching of small crabs is caused by using gill nets with a small mesh size. In this location, the fisherman uses a mesh size of 3 inches. Yu et al. (2024) said that Increased mesh size decreased the capture probability of undersized crabs. The optimal gill net size for crab fisheries has yet to be scientifically established, and the current use of gill nets with different mesh sizes often results inproblems with the bycatch and discarding of small-sized crabs. Small crabs caught cannot be returned to the sea because they are usually damaged when removed from the gill net. These injuries can cause death or delayed death after release into the ocean or affect somatic growth, negatively impacting fishery yields.

Catching undersized crabs, especially ovigerous females, is very harmful to sustainability.Even though the Indonesian government, through the Ministry of Maritime Affairs and Fisheries, has issued regulations limiting the catching of small-size crabs (<10 cm) and prohibiting catchingegg-laying crabs, it seems that these regulations cannot yet be enforced in the crab fishing area inPare-Pare Bay. Digamadulla and de Croos (2023) said in developing countries, the limited humanand financial resources for fisheries management often result in the lack of continuity of fisheriesstatistics, lack of standardized data collection methods, and under-representation of landing sites,which leads Small Scale Fisheries (SSF) to data-poor or data-limited (DLM) fisheries. Management of SSF in tropical developing nations is generally embarrassed by inadequate government funding, lack of political will, open access regimes, numerous scattered landing sites, and shortage of resource users' participation in decision-making.

Apart from the high level of exploitation and the use of non-selective fishing gear such asgill nets, the unique shape of the location is curved inward, making it difficult for crabs to migrateto other areas or not go far from the fishing location. As a result, catching tends to be more accessible, and the number of crabs caught is smaller. This is a big challenge for the crab fishery in Pare-Pare Bay. Fishing for ovigerous crabs is also the biggest threat to the sustainability of crabfisheries in Pare-Pare Bay.

This study found that the fecundity of female blue swimming crabs varied from 107.640 eggs to 1.308.400 eggs (Figure 7). Low fecundity is generally found in petite females about 8 mmin Outer Carapace Width. Kumar et al. (2000) found that a female blue swimming crab can produce 650.000 – 1.760.000 eggs. Haputhantri et al., 2022, also reported low fecundity of blue swimmingcrabs caught in Northern Coastal Waters in Sri Lanka. The lowest fecundity was reported to be around 123,482 eggs from the small size of crabs, and the highest fecundity was around 3,179,928eggs. The carapace width of the *P. pelagicus,* which was used for fecundity estimate, varied from113 mm to 177 mm. Here, whether the carapace width is outer or inner is not explained. Near Pare-Pare Bay, Nurdin, et al., 2022 reported that the fecundity of Blue Swimmer Crab from SpermondeIsland Waters was higher. It varied from 918.091 eggs to 2.095.253 eggs from 91 mm to 140 mmberried females. Kumar et al. (2003) stated that the fecundity of female crabs is size-dependent and increases up to a carapace width of 134 mm and decreases after that. Fecundity increased by 83,9% with an increase of carapace width from 105 mm to 125 mm, implying that a single large female could produce as many eggs as petite females.

Pare-Pare Bay is a semi-enclosed bay with a submarine canyon, a contour that juts inward. The water area of Pare-Pare Bay is 2,778 hectares, with a coastline of Pare-Pare Bay of 34 km, starting from the coastal region of Pare-Pare City, which borders Barru Regency to the coastal areaof Ujung Lero, Suppa District. The depth of seawater is in the range of 0.75 meters in the northernpart, and the middle of the bay has a depth of about 20 meters depending on the tide and water supply from rivers. Pare-Pare Bay receives water from several rivers: the Majannang River, Tae River, Maraulang River, Barangkasanda River, Cakeala River, Sabbamparu River, and Agalacangnge River.

The results of this research reveal that there is a close relationship between the physico-chemical dynamics of water and the spawning season. March is the peak of crab spawning in Pare-Pare Bay, where the water temperature increases and the Current is relatively slow (Table 1). Currie and Hooper (2006) stated that in tropical waters, female blue swimmer crabs are found to carry eggs throughout the year; however, there is seasonal variation in the number of egg-bearing females.

CONCLUSION

Based on the results of this research and the analysis that has been carried out, it can be concluded that the reproductive status of crabs in Pare-Pare Bay is unhealthy. The increasingly petite size of egg-laying females and fishing activities that ignore the capture of egg-laying female crabs seriously threaten the sustainability of the crab population and the crab business in Pare-Pare Bay. Strategic steps are needed to restore the health of crab stocks in Pare-Pare Bay. Monitoring the implementation of the Ministry of Maritime Affairs and Fisheries Regulation, limiting fishingat the peak of the spawning season, stock enhancement through restocking, and hatchery efforts for restocking and cultivation are necessary for the sustainability of this species as an essential business commodity. Apart from that, maintaining a healthy aquatic environment is no less criticalfor stock breeding.

ACKNOWLEDGMENT

This research was financially supported by the Collaborative Fundamental Research Institute for Research and Community Service (LPPM) Hasanuddin University for Fiscal Year 2024 based on contract Number 00309/UN4.22/PT.01.03/2024. This research was also supported by the Crab Council National Fisheries Institute (NFI) through the Smithsonian Environmental Research Center (SERC) in sub-award to Hasanuddin University in 2023. Special thanks go to allstudents of the Faculty of Marine and Fisheries Sciences Hasanuddin University who were recruited in this project as enumerators, namely Namirah Mustari, Nur Azizah, Nurfitria Aulia, Muhammad Saldy, Shinta Puspita Ayu Dunia, Maryam, Ananda Adya, Rani Arini Djamaluddin.

REFERENCES

Aguilar R, Johnson E G, Hines A H, Kramer M A, Goodison M R. 2018. Importance of Blue Crab Life History for Stock Enhancement and Spatial Management of the Fishery in Chesapeake Bay Reviews in Fisheries Science, 16 (1-3):117-124.

Curri D R, Hooper G E. 2006. Blue Swimmer Crab (Portunus pelagicus) Fishery 2004/05. Fishery Assessment Report for PIRSA. South Australian Research and Development Institute (Aquatic Science), Adelaide, RD 03.0274-3.

Digamadulla D S, de Croos M D S T. 2023. Syndication of sink-source and area-based resource management as a remedy to sustain a small-scale fishery: The case study of blue swimming crab fishery from Sri Lanka. Ocean and Coastal Management 244: 106787

Ernaningsih, Danial, Syahrul, Umar M T, Nursyahran. 2024. Population Parameters and Exploitation Ratio of Blue Swimming Crab, Portunus pelagicus (Linnaeus, 1758) fromthe Bawasalo Waters Pangkep Regency, South Sulawesi. Egyptian Journal of Aquatic Biology & Fisheries, 28(3): 285 – 298.

Hamid A, Wardiatno Y, Lumbanbatu D T F, Riani E. 2016. Stock Status and Fisheries Exploitation of Blue Swimming Crab Portunus pelagicus (Linnaeus 1758) in LasongkoBay, Central Buton, Indonesia, Asian Fisheries Science 29 (2016):206-219.

Haputhantri S S K, Bandaranayake K H K, Rathnasuriya M I G, Nirbadha K G S, Weerasekera SJ W W M M P, Athukoorala A A S H, Jayathilaka R AM, Perera H A C C, Creech S.

2022. Reproductive Biology and Feeding Ecology of The Blue Swimming Crab (Portunus pelagicus) in Northern Coastal Waters, Sri Lanka. Tropical Life Sciences Research, 33(2), 155–178.

Kumar M S, Ferguson G, Xiao Y, Hooper G and Venema S. (2000). Studies on reproductive biology and distribution of the BSC (Portunus pelagicus) in South Australia waters. SARDI Research Report Series, 47 pp.

Kumar M S, Xiao Y, Venema S, Hooper G. 2003. Reproductive cycle of the blue swimmer crab, Portunus pelagicus, o¡ southern Australia J. Mar. Biol. Ass. U.K., 83: 983-994.

La Sara, Astuti O, Muzuni, Safilu, 2019 Status of blue swimming crab (Portunus pelagicus) population in Southeast Sulawesi waters, Indonesia. AACL Bioflux 12(5):1909-1917.

Lowerre-Barbieri S K, Barbieri L R. 1993. A New Method Of Oocyte Separation And Preservation For Fish Reproduction Studies (1993). Fishery Bulletin, 91(1), 165-170.

Nurdin M S, Putri A P, Satari D Y, Valentine R Y, Azmi F, Haser T F. 2022. Spawning and Reproductive Potential of Blue Swimming Crab (Portunus pelagicus) at Spermonde Archipelago, Indonesia. Jurnal Biodjati 7(2):199–211.

Ogburn M B, Habegger L C. 2015. Reproductive Status of Callinectes sapidus as an Indicator of Spawning Habitat in the South Atlantic Bight, USA. Estuaries and Coasts,

Tirtadanu, Chodrijah U. 2019 Fishery, population parameters and exploitation status of blue swimming crab (Portunus pelagicus) in Kwandang Waters, Indonesia. AACL Bioflux12(4):1323-1334.

Yao F, Coquery J, Lê Cao K-A. 2012. Independent Principal Component Analysis for biologically meaningful dimension reduction of large biological data sets. BMC Bioinformatics,13:24.

Yu M, Herrmann B, Cerbule K, Liu C, Zhang L, Tang Y. 2024. Effect of gillnet mesh size on the capture probability and capture patterns in the Asian paddle crab (Charybdis japonica) fishery. Heliyon 10 (2024) e25771

AN OVERVIEW ON LEPIDOPTERA INSECTS BIODIVERSITY ORDER IN KORCA REGION

Xhuliana Qirinxhi[1], Eljton Halimi[2], Anila Paparisto[2] , Desareda Mero[1], Elena Hysi[1]

[1] *University "Fan.S. Noli", Korçë, Faculty of Natural Sciences and Human Sciences, Albania*

[2] *University of Tirana, Faculty of Natural Sciences, Tiranë,*

AlbaniaCorresponding author e-mail: xqirinxhi@unkorce.edu.al

ABSTRACT

Studies and research about Lepidoptera (butterfly) are numerous. This is due to the continuous nature of the research, which can never be fully completed. Butterflies are one of the most important groups in various habitats and environments, as well as one of the largest fauna groups. These organisms play a significant ecological role in these environments. The collection of biological material for this paper was conducted during the period of 2021-2022 at various stations in the Korça region, specifically: Drenova, Voskopoja, Dardha, Morava, Polena, and Voskop, among others. We collected biological material from May to September, which coincides with the flight period of these species. During the study, 290 individuals belonging to 25 species were collected. The family with the highest biodiversity of species is Nymphalidae, with 12 species, while the family with the fewest species is Papilionidae, with 2 species. The station with the highest diversity observed was Morava, with 15 species, and the station with the lowest diversity was Voskop, with 3 species.

Keywords: Lepidoptera, ecosystem, insect's, biodiversity, Korça.

INTRODUCTION

The history of Lepidoptera studies in Albania has seen significant developments in understanding and mapping the distribution of these species. The first publications on Lepidoptera in Albania were made in the early 20th century by foreign authors who explored the Albanian territories of that time. This was followed by contributions from both foreign and local authors (Beshkov, 1995; Beshkov and Misja, 1995; Beshkov et al., 1996). The first distribution maps of butterflies in Albania were published by Misja (Misja, 2005). In recent years, there has been a growing interest in studying Albanian Lepidoptera, resulting in new distribution maps for various species (Cuvelier et al., 2018; Verovnik et al., 2013). These studies represent comprehensive efforts across Albania, contributing to the discovery and documentation of numerous butterfly species. Currently, 205 butterfly species have been confirmed for Albania, but it is believed that there are potentially another 10-15 species yet to be identified (Cuvelier et al., 2022, Qirinxhi et al., 2024). A key role in conserving butterfly populations lies in protecting specific habitats that are particularly susceptible to serious human threats (Cuvelier et al., 2022).

In the region of Korçë, the biodiversity of insects of the order is important for the local ecosystem. The Korçë area covers an area of approximately 3711 km² and is geographically located in the southeast of the country. It presents a variety of habitats preferred by Lepidoptera species. The Korçë district has geographic coordinates between 40° 37′ and 40° 57′ northlatitude, and between 21° 4′ and 20° 19′ east longitude (Qiriazi, 1998). The Korçë area is rich in terms of flora and fauna. In this region, we find the presence of almost all plant categories. Despite its limited extension, at very low altitudes above sea level, the Mediterranean scrubland layer extends. More broadly, the layer of scrubland extends along the edge of the brown lands. Above the barren forest lands rise the layers of heaths and halos with dense forests. In the mountain meadowlands, we find shrubs and alpine meadows with a wide variety of grasses and flowers (Dida, 2003).

MATERIAL AND METHODS

Detailed descriptions of the habitat types where Lepidoptera specimens are collected are crucial. This could include data on vegetation composition, dominant plant species, and other relevant environmental factors (Kremen et al., 1993).

Biological material was collected during the years 2021-2022 in the Korçë region during the period from May to September, coinciding with the flight season of Lepidoptera and their life cycle.

a. Study Area

We collected biological material in the Korçë region, specifically at six main stations: Drenovë, Voskopojë, Dardhë, Moravë, Polenë, and Voskop (Figure 1). Drenovë (40.587624 N, 20.788493 E, according to Google Earth Version 7.1) is predominantly characterized by terrain consisting of a combination of fields, gardens, and small forests, creating a suitable habitat for a variety of organisms.

Figure 1. Collection of species photo by

The relief of Voskopojë (40.627708 N, 20.588973 E according to Google Earth Version 7.1) is characterized by hills and low mountains. It is rich in extensive forests and shrubs (Qiriazi, 1998).

The relief of Dardhë (40.517698 N, 20.824692 E according to Google Earth Version 7.1) combines open fields, hills, and dense forests. This area has an average elevation above sea level of about 800-900 meters (Qiriazi, 1998).

The relief of Moravë (40.6270 N, 20.8542 E according to Google Earth Version 7.1) is located east ofKorçë, 30 km long and 10 km wide. The dominant elevations are between 1000-1500 m. Its vegetation cover consists of beech, pine, maple, as well as grasslandplants (Qiriazi, 1998).

The relief of Polenë (41.8445 N, 23.1047 E according to Google Earth Version 7.1)includes a variety of landscapes, from wide and flat fields to low hills and high mountains (Qiriazi, 1998).

The relief of Voskop (40.6050 N, 20.6982 E according to Google Earth Version 7.1) is located at an average altitude of about 1,160 meters above sea level, making it a place with a cool mountain climate during summer and cold winters (Qiriazi, 1998).

b. Collection and Conservation of Material

The collection of material was conducted between 09:00 and 18:00 hours, varying depending on climate and seasons. To minimize damage to captured Lepidoptera specimens, we employed soft nets and careful handling during transfer and storage (Janzen, 2004). Field collection of Lepidoptera individuals was performed using aerial entomological nets made of muslin or nylon, with a diameter of 30 cm and a length of 70-80 cm, attached to a wooden or metal handle of 100 cm (Paparisto & Misja, 2005). The collected material was kept dry during the study period. Before starting the identification process, specimens were relaxed by placing them on an absorbent paper in a standard glass desiccator with a 40 cm diameter reservoir filled with water at 100°C, maintained under vacuum for 24 hours. After relaxation, specimens underwent mounting procedures. The mounted material was dried at room temperature (18- 23°C) for about 3-4 weeks (Paparisto & Misja, 2005). Proper drying techniques are crucial to prevent mold growth and ensure long-term preservation. This involved controlled drying environment with specific temperature and humidity setting (Robinson et al., 1970). During collection, it was crucial to keep detailed records including GPS coordinates, dates, habitat descriptions, photographs of habitats of collected or observed species in the field (Paparisto & Misja, 2005). Responsible collecting practices dictate taking only the minimum number ofspecimens necessary for identification and avoiding over collecting from a population (Gullan & Cranston, 2010). To create collections of Lepidoptera species, individuals were placed in entomological boxes measuring 70 by 60 cm and 10 cm deep, lined with a 1 cm thick layer of cork and covered with 24 sheets of 80 gsm white paper. Adequate spacing between individuals, ranging from 1-2 cm depending on size, was maintained. Specimens were arranged in boxes according to order/family and genus. Data were crucial for understanding new classifications, information about Lepidoptera, and data updates (Walker et al., 1999). Each specimen was accompanied by a label containing the scientific name, place of collection, collection date, and identifier's name. A second label provided supplementary information such as collecting plant or habitat, and a third label was used for identification, containing the specimen's name, identifier's name, and identification date (Walker et al., 1999). To ensure data accuracy, implementing a quality control process is essential. We double-checked specimen identification by a second taxonomist (Turner et al., 2001). Digital photographs were captured of each Lepidoptera specimen. This approach provides a permanent record for future reference and identification purposes (Eliot & Kawahara, 2005). We used Microsoft Excel to organize and explore basic patterns in species diversity, abundance, and potential habitat associations within the Korça region (Magurran, 2013). For long-term preservation, entomological boxes were stored in a cool, dry, and dark location to minimize degradation (Emmel, 1973). Collaboration with museums or research institutions can be beneficial for long-term specimen storage and accessibility (Gullan & Cranston, 2010). Collections were deposited in a museum to prevent issues related to habitat

destruction or minimization (Gullan & Cranston, 2005). Each entomological box containing biological material was labeled with a sequential number and the identifier's name (Paparisto & Misja, 2005).

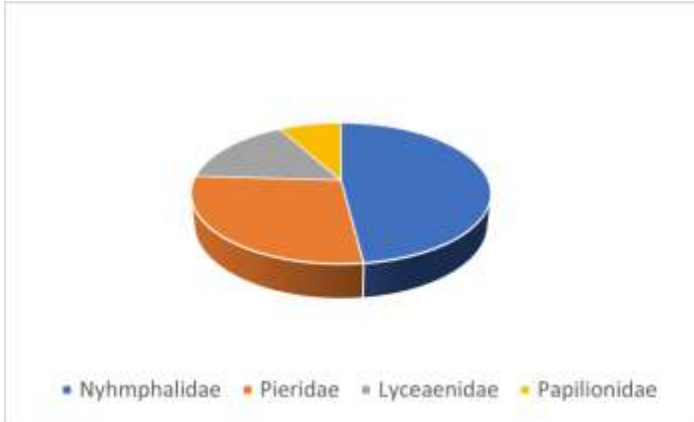
RESULTS AND DISCUSSIONS

This study determines the list of all Lepidoptera species in the Korçë region across their 6 collection stations (Annex 1). From the taxonomic determination of 290 collected individuals in reference to the Korçë area, 25 species were identified distributed across 4 families.

From the analysis of families based on the number of species (Table 1, Graph 1), it results that the family Nymphalidae exhibits the highest species diversity, with 12 species or 48%, followed by the family Pieridae with 7 species or 28%, Lycaenidae with 4 species or 16%, and Papilionidae with 2 species or 8%. Based on monitoring conducted over the past two years, we believe that natural habitats in the Korçë area are most suitable for species of the Nymphalidae family.

Table no. 1. Number of species per families

No.	Family	Specie No x family	Specie % x family
1.	Nyhmphalidae	12	48%
2.	Pieridae	7	28%
3.	Lyceaenidae	4	16%
4.	Papilionidae	2	8 %

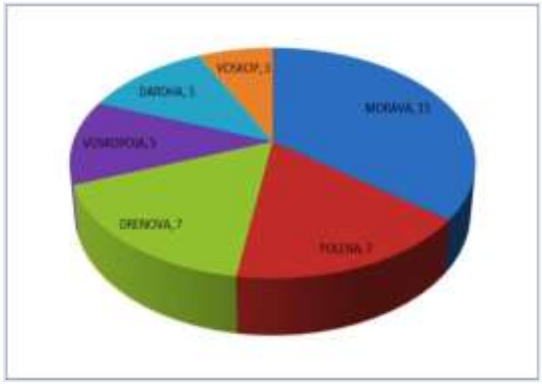


Graph no. 1: Percentage of species per family

From the analysis of stations based on the number of species (Table 2, Graph 2), it is evident that Moravë station has the highest diversity with 15 species, followed by Polenë and Drenovë stations with 7 species each. Next, Voskopojë and Dardhë have 5 species each, while Voskop station shows the lowest diversity with 3 species. Our observations indicate that this station has fewer species due to environmental pollution from industrial and anthropogenic activities such as land excavation, cultivation, and burning, which negatively impact the life cycle of butterflies (Choudhary & Chishty, 2020).

Tabela 2. Number of species by stations

Stations	**Number of species**
Morava	15
Polena	7
Drenova	7
Voskopoja	5
Dardha	5
Voskop	3



Graph no. 2: Number of species per station

The main factor influencing the population of Lepidoptera during our observation, and material collection, was human activity through unregulated construction and environmental pollution (Van Swaay et al., 2012). During our material collection over these two years, it was found that the highest number of butterflies occur in the months of July, August, and September due to humidity, precipitation, and flowering, which influence their numbers (Sharma & Sharma, 2013; Priya & Krishnaraj, 2017).

CONCLUSION

Our study identifies 25 species of Lepidoptera across 6 collection stations. The family Nymphalidae exhibits the highest biodiversity with 12 species, while the family with the lowest biodiversity is Papilionidae with only 3 species. Moravë area shows the highest species diversity with 15 Lepidoptera species, whereas Voskop area exhibits the lowest number of Lepidoptera species with only 3. During the study, it was found that the decline in species numbers is due to unfavorable ecological conditions, habitat disturbance, degradation, isolation, habitat loss, and climate change.

ACKNOWLEDGMENT

This study was conducted with the support of Fan S. Noli University of Korçë, as part of scientific research projects.

REFERENCES

Beshkov, S. 1995. A contribution to the knowledge of the Lepidoptera fauna of Albania. Some findings of a collecting trip in September 1993 (Lepidoptera, Macrolepidoptera). Atalanta. Int J Res Rev. 26(1-2): 365-399.

Beshkov, S., Misja, K. 1995. A contribution to the knowledge of the Lepidoptera fauna of Albania. Some materials from the collection of K. Misja in the Natural History Museum Tirana and some results of the collecting trip of Beshkov during 1992 (Lepidoptera, Macrolepidoptera). Atalanta. Int J Res Rev. 26(1-2): 345-363.

Beshkov, S., Misja, K., Abadjiev, S. 1996. A contribution to the knowledge of the Lepidoptera fauna of Albania. Some results of the collecting in April 1994 and some materials from the collection of the Natural History Museum of Tirana (Lepidoptera, Macrolepidoptera, Heterocera). Atalanta. Int J Res Rev. 27(3-4): 623-648.

Choudhary, N.L., Chishty N. 2020. Comparative study of butterfly between native vegetation and Prosopis juliflora dominated area in Udaipus district, Rajasthan. International Journal of Entomology Research. 5(1):70-73.

Cuvelier, S., Parmentier, L., Paparisto, A., Couckuyt. J. 2018. Butterflies of Albania – Fluturat e Shqipërisë. New surveys, new species and a new checklist (Lepidoptera: Papilionoidea). Phegea. 46: 31-52.

Cuvelier, S., Parmentier, L., Qirinxhi, Xh., Paparisto, A. 2022. Butterflies of Albania new data and going online. Fluturatat e Shqipërisë të dhëna të reja dhe faqja online (Lepidoptera: Papilionoidea). Buletini i Shkencave te Natyres. Tirana university. 32: 5-31.

Dida, M. 2003. State of Forest Tree Genetic Resources in Albania. 62-23.

Eliot, J. N., & Kawahara, A. Y. 2005. Twenty-one new species of Hypolimnas Doubleday (Lepidoptera: Nymphalidae) from the Indo-Australian Region. Zoological Journal of the Linnean Society, 143(1), 1-86.

Emmel, T. C. 1973. Collecting, Preparing, and Preserving Insects. Plants and Gardens Handbook Series: 111. Entomological Society of America.

Gullan, P. J., & Cranston, P. S. 2005. The Insects. An outline of entomology. Oxford, Blackwell Publishing Ltd.

Gullan, P. J., Cranston, P.S. 2010. The Insects: An Outline of Entomology. Wiley-Blackwell.
Janzen, D. H. 2004. Essentials of the Biodiversity Inventory. University of Chicago Press.

Kremen, C., J. L. Liesner, R. W. Montoya, and J. L. Williams. 1993. Landscape connectivity andthe conservation of biological diversity. Biodiversity and Conservation 2(1): 49–61.

Magurran, A. E. 2013. Measuring Biological Diversity. Wiley-Blackwell.

Misja, K. 2005. Fluturat e Shqipërisë. Macrolepidoptera (Rhopalocera,Bombyces & Sphinges, Noctuidae, Geometridae). — Akademia e Shkencave eShqipërisë, Instituti i Kërkimeve Biologjike,Tiranë, 247 p.

Paparisto, A., Misja, K. 2005. Entomological manual for laboratory work ISBN 99943-681-8-4.

Priya, L., Krishnaraj, V. 2017. Studies on butterfly diversity in Adichanalloor Village, Kollam District, Kerala. Journal of Entomology and Zoology Studies. 5(5): 73-81.

Qiriazi, P. 1998. Gjeografia fizike e Shqipërisë" Book. 2 Volumes, Tiranë. 298 pages.

Qirinxhi, Xh., Halimi, E., Paparisto, A., Hysi, E., Buzo, Z. 2024. A Perspective in the Study of Biodiversity of Order Lepidoptera in Albania: Case Study using the MRR Methodology. International Journal of Advanced Multidisciplinary Research and Studies. 4(2):1533-1536.

Robinson, G. S., Ackery, P. R., Kitching, I. J., Leung, G. L., & Miller, T. A. 1970. User's Guide to the Global Taxonomic Database of Lepidoptera (Lepidoptera: GloBIS). Natural History Museum, London.

Sharma, M., Sharma, N. 2013. Nectar resource use by Butterflies in Gir Wildlife Sanctuary, Sasan, Gujarat. Biological Forum- An International Journal. 5: 56-63.

Smart, J. 1940. Insects. Instructions for Collectors, No. 4. A British Museum (Natural History), London.

Turner, W., Harper, J. L., & Rawlinson, P. D. 2001. Population monitoring for biodiversity conservation: a framework for practice. Biology and Environment: Proceedings of the Royal Irish Academy, 101B (3), 115-125.

Van Swaay, C.A.M, Warren, M.S. 2012. Developing butterflies as indicators in Europe: current situation and future options. 22: 5-16.

Verovnik, R., Popović M. 2013. Annotated checklist of Albanian butterflies (Lepidoptera, Papilionoidea and Hesperioidea). - Zookeys 323: 75–89.

Walker, A. K., Fitton, M.G., Vane-Wright, R.I., Carter, D.J. 1999. Insects and other invertebrates. In: Care & Conservation of Natural History Collections, Oxford, Butterworth- Heinemann, p.37-60.

Appendix.

List of species by collection stations:Morava (S1), Polena (S2), Drenova (S3), Dardha (S4), Voskopoja (S5), Voskop (S6)

No.	Species	Family	S1	S2	S3	S4	S5	S6
1.	*Aporia crataegi*	Pieridae		x	x			
2.	*Leptidea duponcheli*	Pieridae	x			x		
3.	*Glaucopsyche alexis*	Lycanidae	xx					
4.	*Coenonympha pamphilus*	Lycanidae	xx		x	x		
5.	*Colias caucasica*	Pieridae	x					
6.	*Colias crocea*	Pieridae	x	x	x	x		
7.	*Lysandra bellargus*	Lycanidae	x					
8.	*Hipparchia alcyone*	Nymphalidae					x	
9.	*Iphiclides podalirius*	Nymphalidae	xx			x	x	
10.	*Issoria lathonia*	Nymphalidae	x	x				
11.	*Polyommatus icarus*	Lycanidae	x					

12.	*Leptidea sinapis/juvernica*	Pieridae	x		x			
13.	*Limenitis reducta*	Nymphalidae					x	
14.	*Argynis paphia*	Nymphalidae					x	
15.	*Melanargia galathea*	Nymphalidae	x					
16.	*Melitaea phoebe*	Nymphalidae	x					x
17.	*Pararge aegeria*	Nymphalidae	x	x				
18.	*Parnassius apollo*	Papilionidae					x	
19.	*Pieris rapae*	Pieridae	x	x				x
20.	*Boloria dia*	Nymphalidae	x					
21.	*Pontia edusa*	Pieridae	xx		x	x		x
22.	*Vanessa atlanta*	Nymphalidae	x					
23.	*Zerynthia cerisy*	Papilionidae	x	x	x			
24.	*Apatura ilia*	Nymphalidae						
25.	*Argynnia pandora*	Nymphalidae						

DATA ON THE FAMILIES NYMPHALIDAE, PIERIDAE, PAPILIONIDAE, HESPERIIDAE, AND LYCAENIDAE OF THE ORDER LEPIDOPTERA IN THEKORÇË REGION (ALBANIA)

Xhuliana Qirinxhi[1], Eljton Halimi[2], Anila Paparisto[2], Elena Hysi[1], Rigela Fero[1]

[1] University "Fan.S. Noli", Korçë, Faculty of Nature Science and Human Science. Korca,Albania

[2] University of Tirana, Faculty of Nature Science.

Tiranë, AlbaniaCorresponding author e-mail:

xqirinxhi@unkorce.edu.al

ABSTRACT

A study was conducted to document the butterfly diversity in the Korçë area from May to September 2022-2023 across four stations: Moravë, Drenovë, Ersekë, and Pirg in the Korçë region, located in the southeastern part of Albania. A total of 241 individuals from the families Nymphalidae, Pieridae, Papilionidae, Lycaenidae, and Hesperiidae were collected, identified into 36 species. Our analysis revealed that the family with the highest species biodiversity is Nymphalidae, while the family with the lowest biodiversity is Lycaenidae. The area with the highest diversity is Drenovë, and the area with the lowest diversity is Pirg.

Keywords: Lepidoptera, Nymphalidae, Pieridae, Papilionidae, Lycaenidae, Hesperiidae biodiversity, ecology, Albania.

INTRODUCTION

The Korçë region covers an area of approximately 3711 km² and is geographically located in the southeast of the country, featuring a variety of habitats favored by Lepidoptera species.The Korçë area has an elevation of 869 meters above sea level, with geographic coordinates of 40° 37′ to 40° 57′ northern latitude and 21° 4′ to 20° 19′ eastern longitude (Qiriazi, 1998). In the mountainous meadowlands, there are alpine pastures and meadows with a wide variety of grasses and flowers (Dida, 2003). Korçë is rich in forests of pine, fir, beech, hawthorn, willow, poplar, and oak. The forested areas host a wealth of endemic vegetation, including medicinal plants suchas wild mallow, savory, chamomile, black juniper, jujube, agrimony, eyebright, and many other species (Belba, 2013).

Nymphalidae represents a family with a significant number of well-known Europeanbutterflies. Among the most distinctive characteristics of this family are the conspicuous colors (Higgins & Riley, 1980). Pieridae is a family with a very large number of species. The species inthis family are generally easy to distinguish, with white or yellow wings and upper surfaces marked with black (Higgins & Riley, 1980). Papilionidae is another family with a considerable number of species that vary greatly in appearance and structural construction. A distinctive characteristic is the single anal vein on the hind wing and the slightly concave inner margin (Higgins & Riley, 1980). Lycaenidae are small or very small butterflies, with males typically having blue, copper, or brown coloring, while females are usually less vibrant. One of the distinguishing features of this family is the hind wings, which have small spots and streaks that are usually identical in both sexes (Higgins & Riley, 1980). Hesperiidae are small butterflies characterized by a broad head, widely separated antennae, and a strong thorax (Higgins and Riley, 1980). Butterflies are considered good indicators of the health of any terrestrial ecosystem and are therefore treated as an important group of organisms for studying environmental and evolutionary processes (Chen et al., 2016).

MATERIAL AND METHODS

The research was conducted during the years 2022-2023 in the Korçë region from May to September. Biological material was collected specifically at four main stations: Moravë, Drenovë, Ersekë, and Pirg (Figure 1). These areas present a great variety in terms of their terrain,climate, altitude above sea level, vegetation cover, and wildlife. Moravë Mountain Station(40.6167° N, 20.6833° E):** Located east of Korçë, it is 30 km long and 10 km wide. This mountain lies between the Devoll plain to the east, the Korçë plain to the west, the Cangonj gorge to the north, and the Kazani Pass to the south (Qiriazi, 1998). Its vegetation cover consists of fir, pine, beech, and herbaceous plants (Qiriazi, 1998). Drenovë Station (40.5667° N, 20.7333° E):** This is a rural area located 4.7 km from Korçë. Its vegetation cover consists of fir, beech, black pine, and herbaceous plants (Qiriazi, 1998). Ersekë Station (40.337° N, 20.678° E):** Located at an altitude of approximately 1,050 meters above sea level. The area is rich in water resources and has fertile soil that favors agriculture, making it well-known for its agricultural andlivestock products (Qiriazi, 1998). Pirg Station (40.600° N, 20.780° E):** Located near the city of Korçë, in the southeast of Albania. The altitude of the Pirg area is around 1,100 meters above sea level (Qiriazi, 1998).

To ensure data comparability across different studies, employing consistent butterfly collection methods is crucial (Van Swaay et al., 2006). This includes using nets of similar size and mesh, along with standardized capture times and weather conditions. Butterflies were captured using entomological nets made of canvas or nylon, with a diameter of 30 cm, a lengthof 70-80 cm, and a handle made of wood or metal, 100 cm long (Paparisto & Misja, 2005). Studies suggest that butterfly activity levels peak during warmer hours with moderate sunlight. Targeting these periods can optimize capture success (Dennis & Sparks, 1991). The capture process began in the morning around 09:00 and continued until 18:00 (Figure 1). Once captured, butterflies should be stored in paper envelopes to minimize damage before pinning (Gullan & Cranston, 2010). This helps to maintain the integrity of their delicate wings and bodies. The collected material was kept dry during the study period.

Before starting the identification process, the specimens are softened by placing them on absorbent paper in a standard glass desiccator with a diameter of 40 cm. The reservoir is filled with water at a temperature of 100°C, and the specimens are kept in a vacuum state for 24 hours. After softening, the specimens undergo the pinning procedure (Paparisto & Misja, 2005). To form the necessary collections for study, butterflies were carefully pinned to avoid damaging their wings, antennae, or legs. For this procedure, entomological pins, 36-38 cm long, were used (Paparisto & Misja, 2005). The pins were inserted vertically into the butterfly's back, between the forelegs and midlegs. For spreading, blocks made of cork material were used (Paparisto &Misja, 2005).

Figure 1. Collection of species photo by @Xh.Qirinxhi

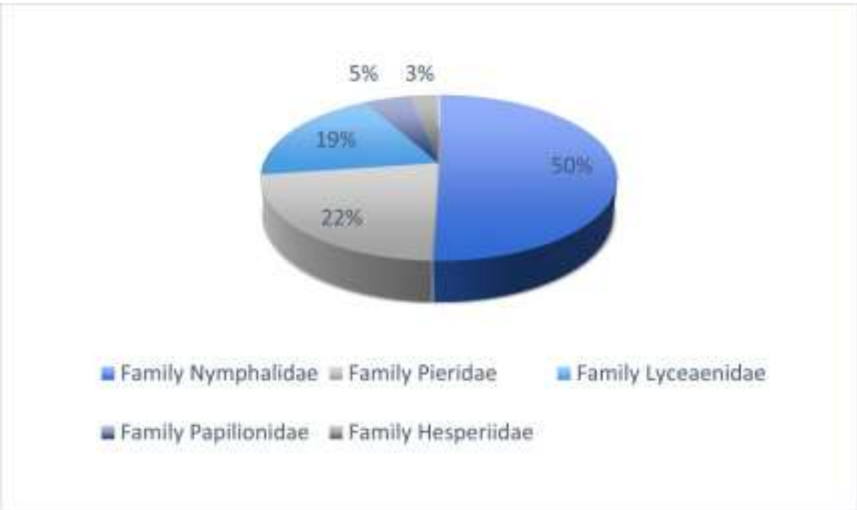
Following pinning, butterflies require a controlled drying period at room temperature to prevent mold growth and ensure long-term specimen preservation (Emmel, 1973). The pinned material is dried at room temperature (18-23°C) for about 3-4 weeks. The dried butterflies are labeled with two tags. One tag indicates the collection location and capture date, while the other displays the scientific name of the species. The butterflies, equipped with the appropriate labels, are placed in collection boxes, typically measuring 40 x 50 cm, which must be sealed hermetically (Paparisto & Misja, 2005). Creating digital images of pinned specimens can be a valuable archiving technique, allowing for wider accessibility of data and facilitating future research (Gullan & Cranston, 2005).

RESULT AND DISCUSSION

This study identifies a list of 241 individuals in the Korçë region, consisting of 36 species distributed across 5 families. From the analysis of families based on the number of species (Table 1, Graph 1), it is evident that the family Nymphalidae exhibits the highest species diversity, with 18 species or 50%, followed by the family Pieridae with 8 species or 22%, Lycaenidae with 7 species or 19%, Papilionidae with 2 species or 5%, and Hesperiidae with only1 species or 3%.

Table no 1. Number of species per family

No.	Family	Specie No x family	Species % x family
1.	Nymphalidae	18	50%
2.	Pieridae	8	22%
3.	Lyceaenidae	7	19%
4.	Papilionidae	2	5%
5.	Hesperiidae	1	3%



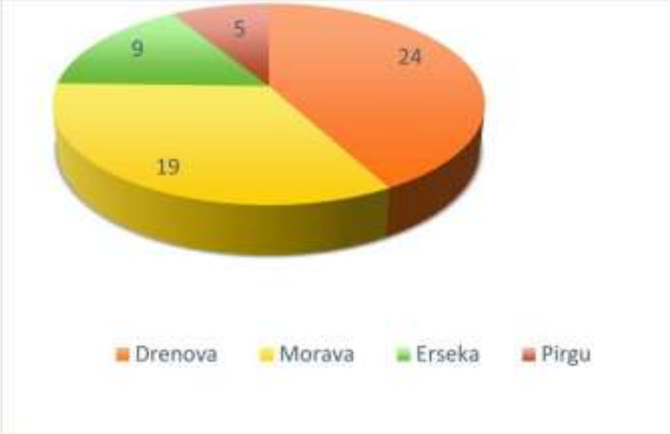
Graph no 1: Percentage of species per family

From the analysis of stations based on the number of species (Table 2, Graph 2), it appears that the station in Drenovës exhibits the highest diversity with 24 species. This is due to the variety of natural habitats and favorable climate conditions that create ideal environments for the survival and proliferation of different species. Following Drenovës, Moravës station has 19 species, and Erseka station has 9 species. The lowest diversity is observed at Pirg station with 5 species, likely due to habitat destruction from human interventions such as construction, intensive agriculture, and deforestation (Sharma & Sharma, 2013; Priya & Krishnaraj, 2017).

Table no. 2: Number of species per station

Stations	No. of species
Drenovo	24
Morava	19
Erseka	9
Pirg	5

Graph no.2: Number of species per station



CONCLUSION

Our study identifies 36 species of Lepidoptera collected from 4 sampling stations. The family Nymphalidae exhibits the highest biodiversity with 18 species, constituting 50% of thetotal species studied, while the family Hesperiidae shows the lowest biodiversity with only 1 species or 3%.

The Drenovë area shows the highest species diversity with 24 Lepidoptera species, whereas the Pirg area exhibits the lowest with only 5 species. During the study, it was observedthat the decline in species numbers is due to unfavorable ecological conditions, habitat disturbance, degradation, isolation, habitat loss, and climate change.

ACKNOWLEDGMENT

This study was conducted with the support of Fan S. Noli University of Korçë, as part of acall for scientific research projects.

REFERENCES

Belba, A. 2013. Disertacion për marrjen e grades shkencore “Doktor i Shkencave”; gjeografia esektorit terciar në rajonin e Korçës, FHF, UT.

Chen, P., J. Awata., H. Matsushita., A. Yang EC., Arikawa, K. 2016. Extreme spectral richnessin the eye of the common bluebottle butterfly, Graphium sarpedon. Front. Ecol. Evol. 4: 1-12.

Dennis, R. L. H., & Sparks, T. H. 1991. Effects of weather on butterfly numbers in Britain andDenmark. Journal of Insect Conservation, 5(1), 173-181.

Dida, M. 2003. State of Forest Tree Genetic Resources in Albania. 62-23.

Emmel, T. C. 1973. Collecting, preparing, and preserving insects (Plants and Gardens HandbookSeries: 111). Entomological Society of America.

Gullan, P. J., & Cranston, P. S. (2005). The Insects. An outline of entomology (Vol. 4). Wiley-Blackwell.

Gullan, P. J., & Cranston, P.S. 2010. The Insects: An Outline of Entomology. Wiley-Blackwell.

Higgins, L.G., Riley, N.D. 1980. A Field Guide to the Butterflies of Britain and Europe(CollinsField Guide)

Paparisto, A., Misja, K. Tiranë 2005. Entomological manual for laboratory work. ISBN 99943-681-8-4

Priya, L., Krishnaraj, V. 2017. Studies on butterfly diversity in Adichanalloor Village, KollamDistrict, Kerala. Journal of Entomology and Zoology Studies. 5(5): 73-81

Qiriazi, P. 1998. Gjeografia fizike e Shqipërisë. Book. 2 Volumes, Tiranë. 298 pages.

Sharma, M., Sharma, N., 2013. Nectar resource use by Butterflies in Gir Wildlife Sanctuary,Sasan, Gujarat. Biological Forum- An International Journal. 5: 56-63.

Van Swaay, C. A. M., Van Strien, A. J., & Termaat, T. (2006). Range contraction and extinctionin the Dutch butterfly fauna since 1890 and the causes of the declines. Journal of Insect Conservation, 10(4), 395-402

STUDY OF HARMFUL AND BENEFICIAL ENTOMOFAUNA ON SUNFLOWER INTHE NORTHEAST BULGARIA

Angel Ivanov[1], Petya Angelova[1]

[1] University of Ruse, Agrarian and Industrial Faculty, Department of Agricultural Machinery, Ruse, Bulgaria

Corresponding author e-mail: aivanov@uni-ruse.bg

ABSTRACT

The aim of the study is to establish the species composition of the harmful and beneficial entomofauna, the dominant harmful species and these entomophages, as well as the species diversity indices. The observations were carried out in the period 2021-2023 in an experimentalfield in the land of Tsar Samuil village. To establish the species composition of the entomofauna, the population density of the dominant harmful species and their entomophages,the classic method - mowing with an entomological bag - was used.

Surveys were carried out during the following periods: outside the sunflower growing season; from the budding phase to the flowering phase of the sunflower and from the floweringphase to the ripening phase of the sunflower.

The study of the entomofauna in the sunflower crop will support the development of goodplant protection practice.

Protecting the sunflower from enemies is a good prerequisite for its cultivation.

Monitoring of the entomofauna showed differences in the quantitative and qualitative composition of harmful and beneficial insects during the three years of the study.

Keywords: sunflower, entomofauna, plant protection practice

INTRODUCTION

The sunflower (*Helianthus annus L.*) is the main and most widespread oilseed crop for Bulgaria and one of the most suitable predecessors for cereal crops.

An important role in realizing the potential of a given variety or hybrid is played by boththe genetic characteristics and the region with the specific soil and climatic conditions in whichsunflower is grown. (Tălmaciu (2018), Lanjar (2014). With the coming changes in the climateand especially with the looming warming, the sunflower is becoming an increasingly preferredcrop, especially from an economic point of view. The inclusion of new varieties and hybrids ofsunflower in the crop rotation allows the diversification of the culture, as well as the reductionof some economic risks.

Sunflower is a crop where short-term drought has no significant economic impact. Of greater importance are the precipitation amounts during separate periods of the vegetation (Altieri M.A. (1999), Jadhav (2011). Regardless of the fact that sunflower is a crop with good ecological plasticity, the choice of a hybrid should be made specifically for agro-ecological regions in order to realize its productive potential to the maximum in the relevant soil and climate conditions.

In Bulgaria, there are more than 70 species of sunflower enemies of economic importance-soil, trunk beetles, plant bugs, crickets and others. In some areas, sunflower cutting reduces yield by about 50-60%. Young plants are attacked by the larvae of the meadow butterfly andby swarming grasshopper species in individual years.

In recent years, as a result of the created extreme conditions, the regulating role of beneficial entomofauna has gained particular importance.

MATERIAL AND METHOD

We carried out the study in an experimental field in the land of the village of Tsar Samuil, commune. Tutrakan during the period 2021 - 2023. We established the species composition of the enemies through standard entomological methods - visual observations, soil excavations, route inspections, mowing with an entomological bag and laboratory determinations. We carried out the surveys during the periods: outside the sunflower growing season; from the budding phase to the flowering phase of the sunflower and from the flowering phase to the ripening phase of the sunflower.

The analysis of these factors during the three years of study (2021 - 2023) shows that theydiffer in terms of precipitation, while the average monthly temperatures are close to those of the multi-year period and fully satisfy the heat requirements of sunflower from sowing to maturation (Figs. 1 and 2).

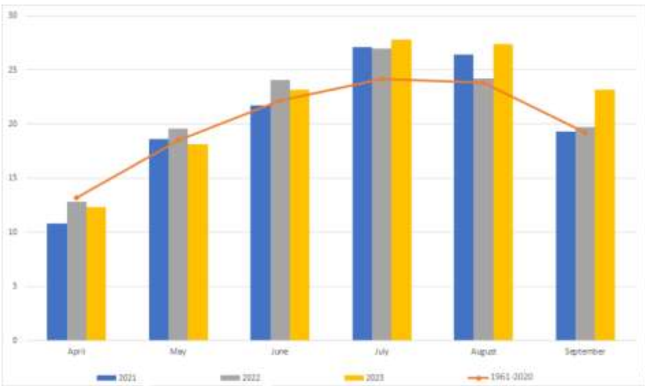


Figure 1. Average monthly air temperature, °C (2021-2023), A multi-year period (1961-2020)

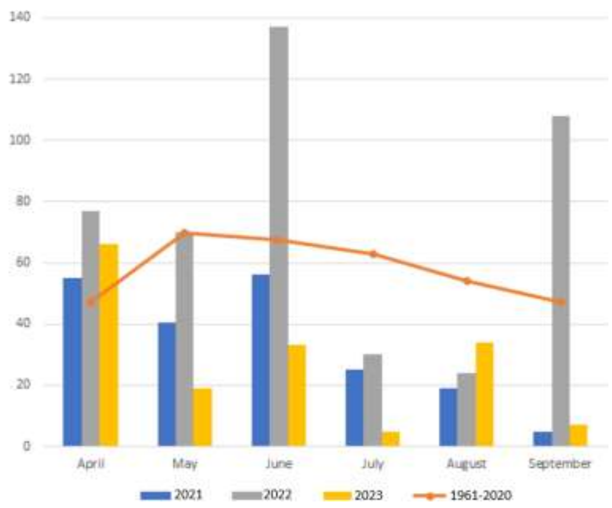


Figure 2. Rainfall, mm (2021-2023), A multi-year period (1961-2020), mm

RESULTS AND DISCUSSION

In the areas intended for sunflower sowing, during soil excavations, the enemies indicatedin table 1 were found.

Table 1: Lists of harmful insects of the sunflower, established in the experimental section

	Scientific name	Order: Family	
1	Lygus pratensis L.	Hemiptera: Miridae	++
2	Lygus rugulipennis Ropp.	Hemiptera: Miridae	++
3	Adelphocoris lineolatus Goeze	Hemiptera: Miridae	+
4	Nezara viridula L.	Hemiptera: Pentatomidae	+
5	Dolycoris baccarim L	Hemiptera: Pentatomidae	+
6	Halyomorpha halys Stal.	Hemiptera: Pentatomidae	+
7	Eurydema oleracea L.	Hemiptera: Pentatomidae	+
8	Spathocera dalmandii Schilling	Hemiptera: Coreidae	+
9	Cercopis vulnerata Ger.	Hemiptera: Cercopidae	+
10	Aphrophora alni Fallen	Hemiptera: Aphrophoridae	+
11	Aphis fabae Scopoli	Hemiptera: Aphididae	+
12	Tanymecus dilaticollis Gyll.	Coleoptera: Curculionidae	+
13	Gastrophysa polygoni L.	Coleoptera: Chrysomelidae	+
14	Chrysolina marginata L.	Coleoptera: Chrysomelidae	+
15	Acanthoscelides aureolus Horn.	Coleoptera: Chrysomelidae	+
16	Helicoverpa armigera Hbn.	Lepidoptera: Noctuidae	+
17	Ectobius vittiventris Costa	Blatodea: Blatellidae	+

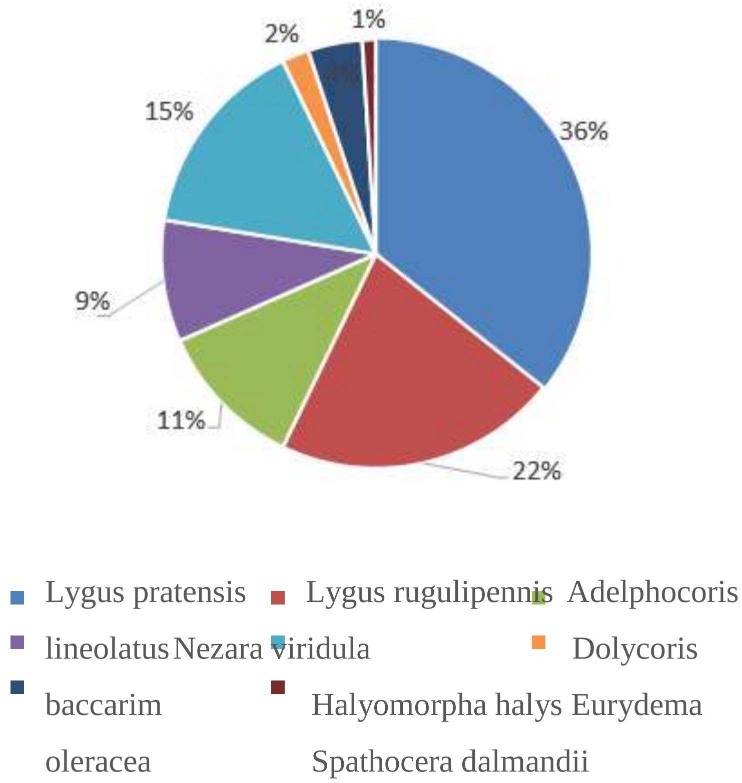


Figure 1. Percentage ratio of bed bug species in a sunflower crop

The collections were made at ten days, starting from sowing to plants harvesting. The collected samples were analyzed in the laboratory at the binocular magnifying glass, determined and separated by species. For each species it was calculated the average density/sqm on the plant entire vegetation period. The results obtained from the collection and determination of thebiological material were calculated and interpreted using some ecological parameters such as: abundance (A%), dominance (D%), constancy (C%) and the index of ecological significance (W%), which highlights the characteristics of the recorded biocenosis. The abundance of species (A%) represents the number of individuals of a species from the catch in a certain place at a certain time. The dominance (D%) shows the participation percentage of each species in the total catches. It is calculated using the formula:

$$D = \frac{AX100}{N}$$

A - species abundance; N - the total number of individuals of all species.

Depending on the obtained values, the species fall within the following classes ofdominance:

D_1-subrecedent species

P<1.0%;D_2-recedent species

P=1.1-2.0%;

D_3-subdominant species P=2.1-

5.0%;D_4 -dominant species P=5.1-

10.0%; D_5 -eudominant species P

>10.1%.

The constancy (C%) represents the species participation proportion in the realization ofthe biocenosis structure. It is calculated using the formula:

$$C = \frac{npA}{Np} X\ 100$$

C - species constancy;

npA - number of samples in which the A species

occurs;Np - total number of collected samples.

Depending on the constancy value, the species are classified as

follows:C_1 - accidental species (1-25%);

C_2 - accessories species (25.1-

50%);C_3 - constant species

(50.1-75%); C_4 - euconstant

species (75.1-100%)

Regardless of the presence of another sunflower crop at a distance of about 1 km from the experimental plot, at no point in the phenological development of the crop did the established enemy species exceed the threshold of economic harm.

The species composition of beneficial insects - predators and parasitoids - is indicated intable 2. The low density of aphids is the probable reason for the establishment of only two species of ladybirds - aphidophages: 7-spot ladybird and variable ladybird. The 22-spotted ladybird, which feeds on mealybug mycelium, was found in the crop only in isolated reports and most likely feeds on mealybug mycelium on some weed species. The predatory bedbug

Nabis sp. was also found on sunflower plants, but its victims (thrips, aphids and other small insects) are mainly on weedy plants. The most abundant predators in May-June were the molluscs Cantharis fusca and Rhagonycha fulva, which have a mixed diet. Adults of the syrphidfly Eopeodes corollae are attracted to flowering plants on whose nectar they feed. No syrphid fly larvae were found in the sparse aphid colonies.

Table 2: Lists of useful insects of the sunflower, established in the experimental section

	Scientific name	Order: Family
1	*Psyllobora vigintiduopunctata* (L.)	Coleoptera: Coccinellidae
2	*Coccinella septempunctata* L.	Coleoptera: Coccinellidae
3	*Hippodamia variegate* Goeze	Coleoptera: Coccinellidae
4	*Cantharis fusca* L.	Coleoptera: Cantharidae
5	*Rhagonycha fulva* (Scopoli)	Coleoptera: Cantharidae
6	*Nabis* sp.	Hemiptera: Nabidae
7	*Eopeodes corollae* Fabricius	Diptera; Syrphidae

As already mentioned, the harmful insect species found on the sunflower were in very low density (Fig. 3).

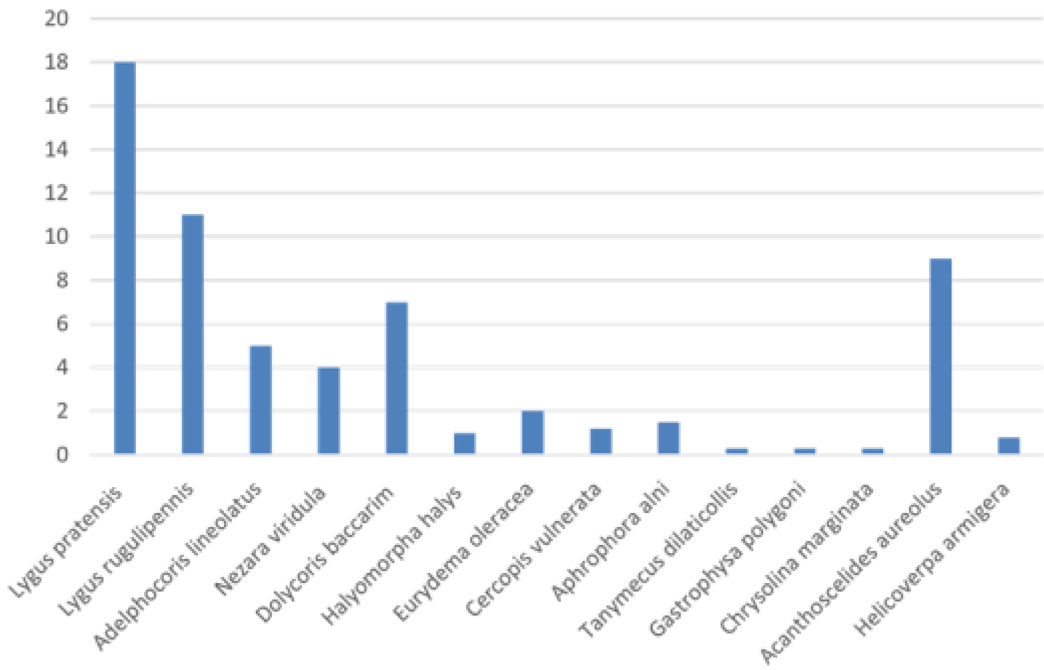


Fig. 3. Average population density of sunflower enemies

In none of the readings from the beginning of the growing season to harvest of the sunflower, there was no enemy that exceeded the defined thresholds of economic harm and necessitated the implementation of a treatment or another plant protection measure.

CONCLUSIONS

Successful pest management depends on the correct identification of the pest, the study of its biology, establishing the population density and comparing it with the economic thresholdof harm, as well as choosing appropriate control methods. The use of integrated management ensures that control decisions will be cost-effective and achieved with minimal negative impacton the environment, as little impact on non-target organisms as possible and with a reduced likelihood of secondary pests. Integrated pest management combines resistant varieties, biological control, and the application of insecticides only when pest populations have reachedeconomically harmful levels. Low-cost components of pest management include crop rotation,modified planting dates, host plant resistance, and conservation of natural enemies (predators, parasitoids, and pathogens).

It is necessary to note that regardless of the many species of entomophages inhabiting sunflower crops and feeding on harmful insects, the regulation of their density by means of natural populations of entomophages is not possible for all enemies, especially for those that are polyphagous. Many occasional insect pests of sunflower can cause high levels of damage, but they are not consistent enough to justify the long-term resources needed to develop integrated management plans. In these cases, efforts are made to use the resistance of the host plants as the main tool to limit their damage. However, in a given area two or three species are considered dangerous pests and the cumulative costs and risks of dealing with them is anincentive to develop more detailed control plans.

REFERENCES

Altieri M.A. (1999). The ecological role of biodiversity in agroecosystems. Agriculture, Ecosystems & Environment. 74: 19-31.

Free JB. (1993) Insect Pollination of Crops. Academic Press, London, UK, 125-142.

Jadhav J.A., Sreedevi K., Rajendra Prasad P. (2011). Insect pollinator diversity and abundancein sunflower ecosystem. Current Biotica. 5(3): 344-350.

Jones G.A., Gillett J.L. (2005). Intercropping with sunflowers to attract beneficial insects in organic agriculture. Florida Entomologist. 88(1): 91-96.

Jajor E., Mrówczyński M. (ed.). 2017. Integrated sunflower protection methodology for producers.Institute of Plant Protection — National Research Institute, Poznań, pp. 156

Lanjar A.G, Solangi A.W., Khuhro S.A., Jiskani R.H., Bukero A., Nissa Rais M.U. 2014. Effect of mix cropping of mustard and sunflower on insect diversity. Science International. 26(4): 1601-1606.

Nechita, A. G., Roșca, I. (2010). Research regarding species of Neuroptera fauna from maize agro ecosystem. Scientific Papers - University of Agronomic Sciences and Veterinary Medicine, Bucharest, Series A, Agronomy, 53, 435-441.

Tălmaciu, M., Herea, M., Tălmaciu, N., Mocanu, I. (2018). New contributions to the knowledgeof the Coleoptera species from some cereal crops. Geo Conference: SGEM: Surveying Geology & mining Ecology Management, 18, 667-673

COMPARATIVE TESTING OF MID-EARLY HYBRIDS CORN FOR GRAIN, CULTIVATED UNDER NON-IRRIGATED CONDITIONS IN THE NORTHEASTBULGARIA

Angel Ivanov[1], Dimitria Ilieva[1], Petya Angelova[1]

[1] *University of Ruse, Agrarian and Industrial Faculty, Department ofAgricultural Machinery, Ruse, Bulgaria*

Corresponding author e-mail: aivanov@uni-ruse.bg

ABSTRACT

The experiment was conducted through the period 2021-2023 in the Northeast region of Bulgaria. The test was conducted in the block method in four repetitions with the size of the experimental plot - 20 m^2. The tested corn hybrids are mid-early (FAO 300-399).

The aim of the study was to establish the elements of productivity and the yield of mid-early maize hybrids, cultivated for grain under non-irrigation in NorthEast Bulgaria. All the stages of the established technology for maize growing were followed. The grain yield is determined with standard grain moisture of 13%. The indices; length of the cob (cm), number of the row per cob, number of the grains per row, mass of the cob, mass of the grains per cob (g) thousandkernel weight (g), test weight (kg), and grain yield (kg/da) were determined. The analysis of the results showed that the production possibility of hybrids maize is determined to a great degree by the meteorological conditions of the year mostly by the precipitation quantity.

Keywords: maize, hybrids, elements of productivity, yield of grain

INTRODUCTION

Maize (*Zea mays L.*) is one of the most important cereal crops grown in the world and plays a key role in the agriculture and economy of Bulgaria. In the north-eastern part of the country, where the climatic conditions are characteristic with their specifics, growing maize isa challenge, especially in non-irrigated conditions. Changes in climatic conditions and the increasing frequency of drought periods require farmers to select suitable hybrids that guaranteestable yields and high grain quality with minimal irrigation resources.

In this context, the testing of early maize hybrids for grain is essential. Early hybrids offeran optimal balance between growing season and yield and free up areas for tillage in time for the following economic year. This makes them suitable for the conditions in North-Eastern Bulgaria, where significant variations in temperatures and rainfall are observed.

The creation and implementation in practice of new corn hybrids, as well as their cultivation under different agro-ecological conditions, are the subject of many scientific studies.(Valchinkov et al., 2003, 2005; Genova, 2005; Genova, 2005; Yordanov, 2006; Angelov, 1995).

The elements of high yield and quality in agricultural crops are a complex of interrelated factors such as: correct crop rotations, quality soil treatment, selection of suitable varieties or hybrids for the specific agro-ecological region, as well as the use of high-quality seeds (Valkova, 2007; Kirchev, 2001; Angelova, 2022, Mohamed et al. 2008).

The selection of the most suitable hybrids for each region in accordance with the conditions and cultivation technology leads to obtaining high results and ensuring stable yields.

The purpose of the study is to determine the elements of productivity and grain yield of early hybrids of corn grown under non-irrigated conditions in North-Eastern Bulgaria.

MATERIAL AND METHOD

The trial covers the period 2021-2023, and it includes key parameters such as cob length,number of rows in the cob, number of grains in a row, number of grains in a cob, mass of grainsin a cob and grain yield. Through a comparative analysis of these indicators, the most suitable hybrids for cultivation in the area are established, which will contribute to a more efficient useof available resources and an increase in yields.

This research will not only provide important data for agronomists and farmers, but will also help to optimize breeding work and improve agricultural practices in the area. The ultimategoal is to increase the resistance of corn under non-irrigated conditions and to increase the competitiveness of Bulgarian agriculture on the international market.

The experimental work was carried out during the period 2021-2023, on a soil type of slightly leached chernozem in the area of the land of the village of Tsar Samuil, Silistra district

- North-Eastern region.

The experiment was set up according to the block method in four repetitions with the sizeof the experimental plot 20 m^2 after predecessor wheat. Maize is grown according to traditionaltechnology.

For the purposes of the study, 5 early hybrids of corn from the AQUAmax® line, of the company "Corteva agriscience" - P9363 (FAO 340), P9300 (FAO 360), P9241 (FAO 370), P9610 (FAO 370), P9415 (FAO 380) were included), grown according to generally accepted technology under non-irrigated conditions for the area. P9300 was used as the standard, being the most widely grown in the area.

Sowing was carried out with previously disinfected seeds (Lumipoza 625 FS), in the period 10 - 12 April at a seeding density of 7200 plants/da. Pre-sowing mineral fertilization with NPK 16/8/10 of the Yara Milla TRIPLE company was applied at a rate of 20 kg/day.

To fight against annual and perennial wheat and broadleaf weeds, in phase BBCH 12-19(dissolution of the second leaf - ninth leaf), the herbicide Principal Gold - 48 g/da (combinationof a.v. Nicosulfuron, Rimsulfuron, Dicamba) was used and adjuvant 0.1% Vivolt 90.

The indicators were reported: cob length (cm), number of rows in a cob, number of grainsin a row, number of grains in a cob, mass of grains in a cob (g) and grain yield (kg/da).

The data on the obtained yields were processed mathematically by the method of varianceanalysis, and the differences between the variants were established by Duncan's multiple rank test SPSS for Windows, v 9.00; Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The main climatic factors determining the growth, development and productivity of corn are temperatures and precipitation, their combination and distribution during the growing season.

The analysis of these factors during the three years of study (2021 - 2023) shows that theydiffer in terms of precipitation, while the average monthly temperatures are close to those of the multi-year period and fully satisfy the heat requirements of maize from sowing to maturation(Figs. 1 and 2). Of the three years of the studied period, 2022 was the most favorable in terms of moisture, during which the highest yields were recorded.

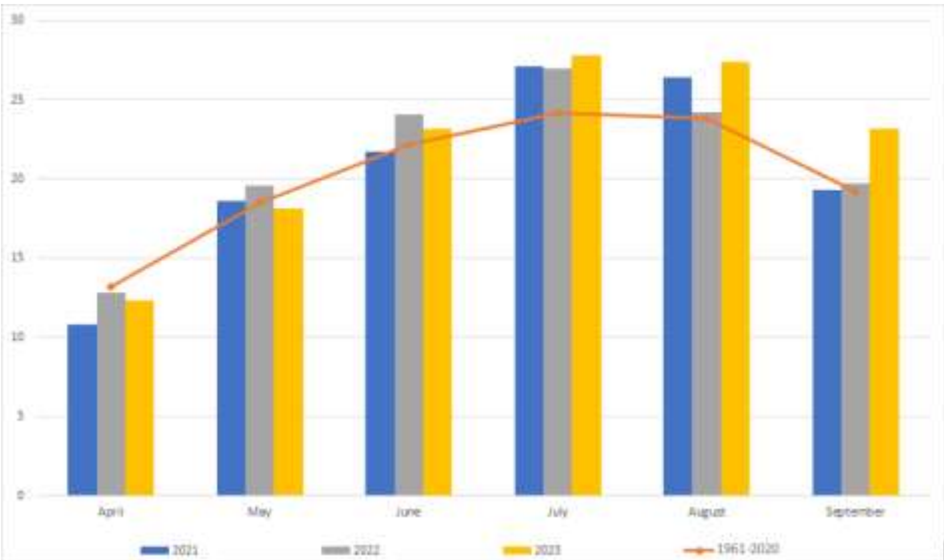


Figure 1. Average monthly air temperature, °C (2021-2023), A multi-year period (1961-2020)

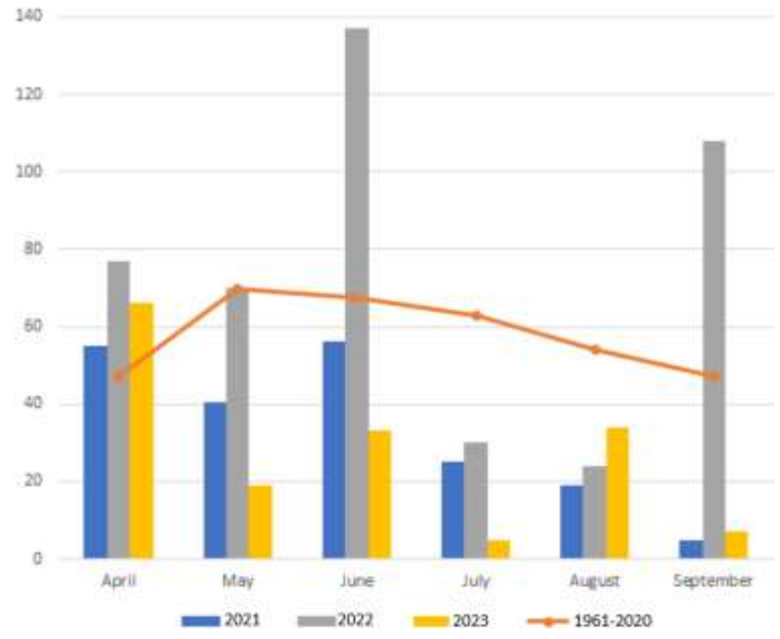


Figure 2. Rainfall, mm (2021-2023), A multi-year period (1961-2020), mm

The established data on the structural elements of yield are indicated in Table 1. They show that these indicators change under the influence of weather conditions during the years ofstudy. The highest values of the main structural elements of production were reported in the economic year 2022, followed by those obtained in 2021, and the lowest in 2023.

Table 1. Structural elements of the yield

Indices	Years of stady	Hybrids				
		P9300 St (FAO 360)	P9363 (FAO 340)	P9241 (FAO 370)	P9610 (FAO 370)	P9415 (FAO 380)
Length of the cob, cm	2021	22,90	21,40	20,90	22,00	20,50
	2022	22,90	22,50	20,70	22,90	21,80
	2023	21,20	21,00	19,40	21,10	20,10
	Average	***22,30***	***21,60***	***20,30***	***22,00***	***20,80***
Number of the row per cob	2021	17,10	16,40	14,70	17,90	15,40
	2022	17,90	16,90	15,20	18,00	16,00
	2023	17,40	16,30	14,60	18,00	15,30
	Average	***17,50***	***16,50***	***14,80***	***18,00***	***15,60***
Number of the grains per row	2021	40,50	39,00	39,10	39,50	38,50
	2022	38,50	38,40	37,00	38,00	38,00
	2023	38,50	39,00	38,40	38,50	38,00
	Average	***39,50***	***38,80***	***38,20***	***38,70***	***38,20***
Number of the grains per cob	2021	577,10	533,00	479,00	589,20	494,10
	2022	574,30	540,80	468,70	570,00	506,70
	2023	558,30	529,80	467,20	577,50	484,50
	Average	*569,90*	*534,50*	*471,60*	*578,90*	*495,10*
Mass of the grains per cob, g	2021	182,30	170,70	156,30	217,30	169,30
	2022	188,40	181,60	156,50	216,00	179,70
	2023	166,60	144,90	141,30	194,50	153,40
	Average	***179,10***	***165,70***	***151,40***	***209,30***	***167,50***

In 2022, the length of the cob varies from 20,7 cm in hybrid P 9241 to 22,9 cm in P 9610,while in 2023 the values of this indicator are significantly lower and are in the range of 19,4 cmin hybrid P 9241 up to 21,2 cm for the standard, and in 2021 from 20,5 to 22,9 cm. On averagefor the research period, regarding the feature cob length, the results show that the highest valueswere recorded for the standard P 9300 – 22,3 cm and hybrid P 9610 – 22,0 cm, and the lowestin hybrid P 9241 – 20,3 cm.

The number of rows in the cob is a genetically determined trait (Genov, 2005, Angelova2022). The reported values from 15,4 to 17,9 pcs. in 2021, from 15,2 pcs. up to 18 pcs. in 2022and from 14,6 pcs. up to 18 pcs. in 2023 show that this parameter, compared to cob length, is less affected by conditions during the years of study. On average, for the study period, higher values of the indicator number of rows in a cob were reported for hybrids P 9610 and P 9300. The hybrid P 9610 has 18 rows in a cob and exceeds the standard by 2,9 %. All other hybrids have lower values.

Regarding the indicator number of grains in a row, the highest values were reported in 2021, from 38,5 for hybrid P 9415 to 40,5 for the standard. The remaining two years are characterized by lower values of 37,0 pcs. to 38.5 in 2022 and from 38.0 to 39,0 pcs. in 2023. On average for the research period, the highest values were reported for hybrid P 9300 – 39,5 pcs., and the lowest 38,2 pcs. at P 9241 and P 9415.

The indicator of the number of grains in a cob in the tested hybrids changed over the yearsand varied from 479,0 in P 9241 to 589,2 pcs. at P 9610, from 468,7 to 574,3 pcs. in 2022, andfrom 467,2 to 577,5 pcs. in 2023. During the three years of the study, the standard hybrid P9300and hybrid P9610 have reported higher values compared to the other hybrids. On average for the period, hybrid P9610 exceeded the standard by 1,6%.

Table 2 Grain yield, kg/da

	Grain yield, kg/ da – year						Average	
Hybrids	2021		2022		2023		kg/da	%
	kg/da	%	kg/da	%	kg/da	%		
P9300 st.	**691**	**100**	**793**	**100**	**631**	**100**	**705.0**	**100**
P9363	707***	102.00	778	98.10	634	100.4	706.33	100.2
P9241	599	86.66	765	96.46	658***	104.2	674.0	95.60
P9610	613	88.87	822***	103.60	597	94.61	677.33	96.07
P9415	603	87.26	889***	112.10	678***	107.4	723.33	102.6
GD 5 %	6,1		5,5		5,7		91,8	
GD 1 %	8,6		7,7		8,0		133,5	
GD 0.1 %	12,1		10,9		11,3		200,6	

The mass of grains in the cob is one of the most important structural elements of yield and determines to some extent the productivity of maize hybrids (Kirchev, 2016). The values of this indicator for all studied hybrids were the highest in 2022 and ranged from 156,5 g for hybrid P 9241 to 216,0 g for P 9610, while in 2021 they ranged from 156,3 g to

217,3 g and from 141,3 years to 194,5 years in 2023. On average for the period, the highest values were reported for hybrid P 9610 – 209,3 years and exceed the standard by 16,9%.

The obtained results for grain yield from the tested corn hybrids show that both the elements of productivity and the values of this indicator change depending on the weather conditions during the years of the experiment (table 2).

In 2022, the vegetation rains that fell, their good distribution and in combination with average monthly temperatures favor obtaining higher grain yields compared to 2021 and 2023.In 2022, hybrid P 9415 realized the highest yield of 889 kg/da and exceeds the standard by 12,1%, followed by hybrid P 9610 – 3,4%, and the lowest was recorded at P 9241 – 765,0 kg/daor by 3,5% lower than the standard.

In 2021, yields for individual hybrids ranged from 599.0 kg/da for P 9241 to 707.0 kg/dafor P 9363, which exceeded the standard by 2.0%. The difference is proven at P 0,1%. Lower yields were recorded for the remaining hybrids.

The uneven distribution of precipitation during the 2023 growing season, and especially its shortage during the grading and dehulling of corn, are the reason for reported lower grain yields. In the tested hybrids, they vary from 597,0 to 678,0 kg/da. During the year, the highestyields were reported by the hybrids P 9241 658,0 kg/da and P 9415 (678,0 kg/da), with the differences compared to the standard being proven at P 0,1%.

On average for the research period 2021 - 2023, of the tested hybrids, the highest yieldinghybrid was P 9415 – 723,33 kg/da. It exceeds the standard by 2,6%, and the lowest yielding hybrid is P 9241 (674,00 kg/da).

The dispersion analysis of grain yield shows a strong statistically proven influence of bothyears (72%) and hybrids (46%) on the variation of the values of these indicators (table 3). Theweakest influence (18%) was shown by the interaction between the two factors (hybrid x year).

Table 3 Analysis of variance for grain yield for the period 2021-2023

Source of Variation	Sum of Square	DF	Mean Square	Sig of F	Degree of influence, %, η^2
Main effects	620549070	1	70023778.76	0.000	
Hybrids	15147003	2	5687506.06	0.000	46
Years	314244816	4	4771566.66	0.000	72
Hybrid xYear	2587967	8	318495.37	0.000	18
Residual	116048	45	243378.08		

SV – *Source of variation*; SS – *Sum of Square*; DF – *Degree of influence*; MS – *Mean Square*; F – *relationshipbetween quantities*; η^2 - *Degree of influence of the factor.*

CONCLUSIONS

The productivity of the tested early hybrids of corn in the Northeast region is determinedto a large extent by the weather conditions of the year and especially by the amount and distribution of precipitation during the growing season.

The hybrid P 9300 - standard and the hybrid P 9610 stand out with the highest values of the structural elements, and the hybrid P 9241 with the lowest.

On average for the research period (2021-2023), a higher grain yield was realized by thehybrid P 9415 – 723,33 kg/da, followed by the hybrid P 9363 – 706,33 kg/da, standard P 9300

– 705,00 kg /da, and the lowest P 9241 – 674,00 kg/da.

From the studied early hybrids of corn for the North-Eastern region, the cultivation of hybrid P9415 is recommended, as it has a higher yield in years with different climatic conditions.

REFERENCES

Angelov, K., E. Lalova, P. Petrov, 1995. Ecological studies of maize hybrids. – Plant science, 5, 36-38.

Angelova P., G. Tassev2022, Basics of Scientific Research in Agronomy, Sofia (371-374)

Bommert P, Nagasawa NS, Jackson D (2013b) Quantitative variation in maize kernel row number is controlled by the FASCIATED EAR2 locus. Nat Genet 45:334–337. https://doi.org/10.1038/ng.2534

Bonea, D., Urechean, V. (2020). Response of maize yield to variation in rainfall and average temperature in central part of Oltenia. Romanian Agricultural Research, 37, 1–8

Delibaltova, V. (2018). Comparative study of grain maize hybrids in the region of north-east Bulgaria. IX International Agriculture Symposium. "Agrosym2018". Jahorina, 4-7 October, Bosnia and Herzegovina, 139–145.

Dimitrova, M., Dimova, D., Zhalnov, I., Zorovski, P., Zhelyazkov, I., Valcheva, E., Popova, R.(2013). The influence of new herbicides on the growth and some structural elements of the yield of fodder maize. Scientific Papers Series A. Agronomy, 56, 226–230.

Genova, I., M. Genov, 2005. Knezha 512 – a new mid-late hybrid. – Plant science, 42, 209- 214.

Genov, M., I. Genova, 2005. Knezha 683A – a new achievement of modern Bulgarian corn selection. – Plant science, 42, 303-307.

Yordanov, G., 2006. Knezha 703 WX – a new hybrid with modified endosperm for grain – Plant science, 43, 228-230.

lchovska, M. (2017). Heterosis and Degrees of Dominance of Grain Yield and Grain Yield Elements in Maize Hybrids in Different Groups of Ripeness.Agricultural Science and Technology, 9(1), 10–15.

Kandil, E. (2013). Response of some maize hybrids (Zeamays) to different levels of nitrogenousfertilization. Journal of Appllied Sciences Research, 9(3).

Kirchev, H. (2016). Comparative study of early and mid-early grain maize hybrids in the conditions of Southern Dobrogea. Research Journal of Agricultural Science, 48(1), 63– 69.

Mohamed, S., A. Sawsan, M. Dalia, (2008). Improving maize grain yield and its quality grown on a newly reclaimed sandy soil by applying micronutrient, organic manure and biological inoculation. – Research Journal of Agriculture and Biological Sciences, 4(5), 537-544

Muneeb, K., Kamran, K., Sami, U.A., Nawab, A., Muhammad, M.A., Hazrat, U., Muhammad, O.I. (2017). Yield performance of different maize (Zea mays L.) genotypes under agro climatic conditions of Haripur. Int.J. Environ. Sci. Nat. Res., 5(5), 555–572.

Popova, R., Zhalnov, I., Valcheva, E., Zorovski P., Dimitrova, M. (2012). Estimates of environmental conditions of Soils in Plovdiv region in applying the new Herbicides for weed control in major Field crops. Journal of Central European Agriculture,13(3), 595– 600.

Popova, Z., Ivanova, M., Pereira, L., Alexandrov, V., Kercheva, M., Doneva, K., Martins, D. (2015). Droughts and climate change in Bulgaria: assessing maize crop risk and Irrigation requirements inrelation to soil and climate region. Bulgarian Journal of Agricultural Science, 21(1), 35–53.

Tasev G., S. Madjov, T. Delikostov, M. Georgieva, P. Ivanova, (2022) Statistical methods for the analysis of experimental results of scientific research, Sofia (522-546)

Valkova, V., 2007. Phenotypic characteristics and elements of maize hybrids KN 625, KN M 625. AU - Plovdiv, LII, 119-124.

Valchinkov, S., A. Popov, P. Petrov, P. Valchinkova , 2003. Maize hybrids Knezha M 625., Stara Zagora, I, 70-73.

Valchinkov, S., A. Popov, P. Hristova, P. Petrov, 2005. Maize hybrids Knezha 517. – Plant science, 42, 21-24.

Yankov, P., Drumeva, M., Plamenov, D. (2014). Variations of maize yield and some quality indices of grain depending on the type of main soil tillage. Agricultural Science and Technology, 6(2), 184–186.

GENETIC DIVERSITY OF BALI POLLED CATTLE DEVELOPED IN SMALLHOLDERFARMS USE THE POLYMERASE CHAIN REACTION-RANDOM AMPLIFIED POLYMORPHIC DNA (PCR-RAPD)

Sudirman Baco[1], Ratmawati Malaka[1], Hasbi[1], Herry Sonjaya[1], Ubaidillah[2]

[1] Department of Animal Production, Faculty of Animal Science, Hasanuddin University, Makassar, South Sulawesi, Indonesia, 90245

[2] Under Graduate Student of Animal Husbandry, Faculty of Animal Science, Hasanuddin University, Makassar, South Sulawesi, Indonesia, 90245

ABSTRACT

The study was conducted at two locations in South Sulawesi, Barru and Bone Regencies. Balipolled cattle from local horned Bali cattle, which are starting to develop in South Sulawesi. The study aims to evaluate the diversity and distance genetics levels in the two development areas. Sample Cow blood has been taken, which is Bali polled / hornless from the results of mating between horned Bali cattle female and Bali polled bull; Bali cattle have horns from results mating between horned Bali cattle female with horned Bali cattle bull, and horned Balicattle with Bali Polled /hornless bull. The technique is to take blood through the jugular vein using a tube venojet. Cattle blood samples from two areas have their DNA extracted, then amplification with the PCR-RAPD method uses six primers. Then, PCR results are visualizedwith electrophoresis. Diversity genetic in and between populations and phylogenetic trees is also analyzed. Diversity and differences genetically were analyzed statistically based on the *Band Sharing Frequency* (BSF) mark. Correlation of genetics between Bali *polled* cattle withBali cattle horns using The Program Software Phylogenetics Trees (MEGA 5.0). The Mark ofBSF for Bali polled cattle between individuals in the population of the district of Bone and Barru ranged from 0.8008 to 0.9025 and 0.840 to 0.9043, respectively. Meanwhile, for hornedBali cattle, BSF values ranged between 0.5745 - 0.7917 and 0.7929 - 0.9721. The genetic distance between Bali cattle populations was 5% - 20% in both livestock development areas. The results showed that the genetic diversity of Bali polled cattle was low compared to Bali cattle with horns. The genetic relationship between Bali cattle and both development areas is still near.

Keywords: Bali polled cattle, genetic diversity, genetic distance, PCR-RAPD, smallholderfarm

INTRODUCTION

Bali cattle have a strategic role in advancing the economy, opening fieldwork, and fulfilling animal protein requirements for Indonesian society. The development of Bali cattle is centered in traditional communities with low levels of livestock ownership; around 98% of the Bali cattle population is distributed among traditional breeders with extensive rearing systems. One of the variants of Bali cattle that is starting to be developed in South Sulawesi is the Bali Polled cattle. This cattle does not have horns so that it is easier to maintain and does not cause risk to other cattle. in 2016, Bali polled cattle began to be developed at the Maiwa Breeding Center, Hasanuddin University in the Enrekang Regency. The Developing Program of the polled Bali Cattle was a collaboration with the Government of the Directorate General Innovation Strengthening, Ministry of Research, Technology and Higher Education. The research and development is a priority program development in Animal husbandry. In 2020, Bali polled cattle were made superior local beef cattle that must be produced and developed inthe livestock farming community. Since then, the production of frozen semen from Bali polledcattle has been massively increased by collaborating between Hasanuddin University, the National Research Agency and the South Sulawesi Provincial Government and several regionalgovernments in South Sulawesi, including Barru and Bone Regencies.

In its development, polled Bali cattle were developed together with horned Bali cattle that exist in the livestock farming community with limited patterns of livestock ownership (SmallholderFarm). Parent cattle used in breeding in the field use Horned Cattle Bali, owned by the breeder,with application technology insemination artificial (IB). Morphologically, it seems that the Balipolled offspring have good performance, but the genetic potential for breeding on people's farms with the Smallholder Farm system like pure Bali cattle (horned) have not yet been recorded. Therefore, research is needed to conduct a study genetic diversity analysis both within and between polled Bali cattle and pure Bali cattle (horned) populations at the two cattledevelopment locations. The specific target that want to achieve is to obtain information about the relationship between environmental factors, phenotypes and genotypes so that superior cattle seedstock can be selected, which have high productivity and fertility levels [1] and can then be utilized by smallholder farmers in South Sulawesi, especially plasma farmers for breeding to support the improvement of the genetic quality of local Bali polled cattle as the forerunner of new superior national beef cattle. One method to reach the above-mentioned objective is DNA analysis using polymerase chain reaction-random amplified p*olymorphic DNA (*RAPD-PCR). This method can analyze the diverse of genomes of species as quickly and efficiently as possible applied to poultry or animals. Specifically, research was designed toparticipate in priority programs on the field farm that enhance the productivity of local cattle, significantly increasing the quality of the seed stock of Bali polled cattle through genetic and molecular studies to improve the genetic quality of local cattle nationwide [2];[3](Figure 1).

Figure 1. Polled Bali Cattle Bull has a Body Weight Reaches 500 kg at the Age of 4.5 Years,and Cows Female *Polled*

This Bali cattle variant, Bali polled cattle, has begun to be developed and even becomea national priority in the development of superior local cattle in Indonesia. Diversity is a normalphenomenon in living things. In principle, diversity genetics studies aim to study individuals' genetic composition within or between Bali cattle populations, polled Bali cattle, and horned Bali cattle.

RESEARCH METHODS

Blood Sample

Blood samples were taken from the jugular vein of Bali cattle in two rearing areas, namely Bone Regency and Barru Regency. 27 and 24 heads of Bali cattle were taken, respectively, ineach region. DNA samples collected from selected cattle from each different rearing location were taken based on the offspring group, that is, Bali polled / hornless as a result of mating between horned Bali cattle cows and Bali polled cattle bull, Bali cattle have horns from resultsof mating between Bali cattle cow with horned and horned Bali cattle bull, and horned Bali cattle from the results of mating between horned Bali cow with Polled /hornless bull. A schemeof the research flow can be seen in Figure 2. DNA was extracted from blood using standard methods, and PCR amplification was carried out using the techniques of [4] and [5]

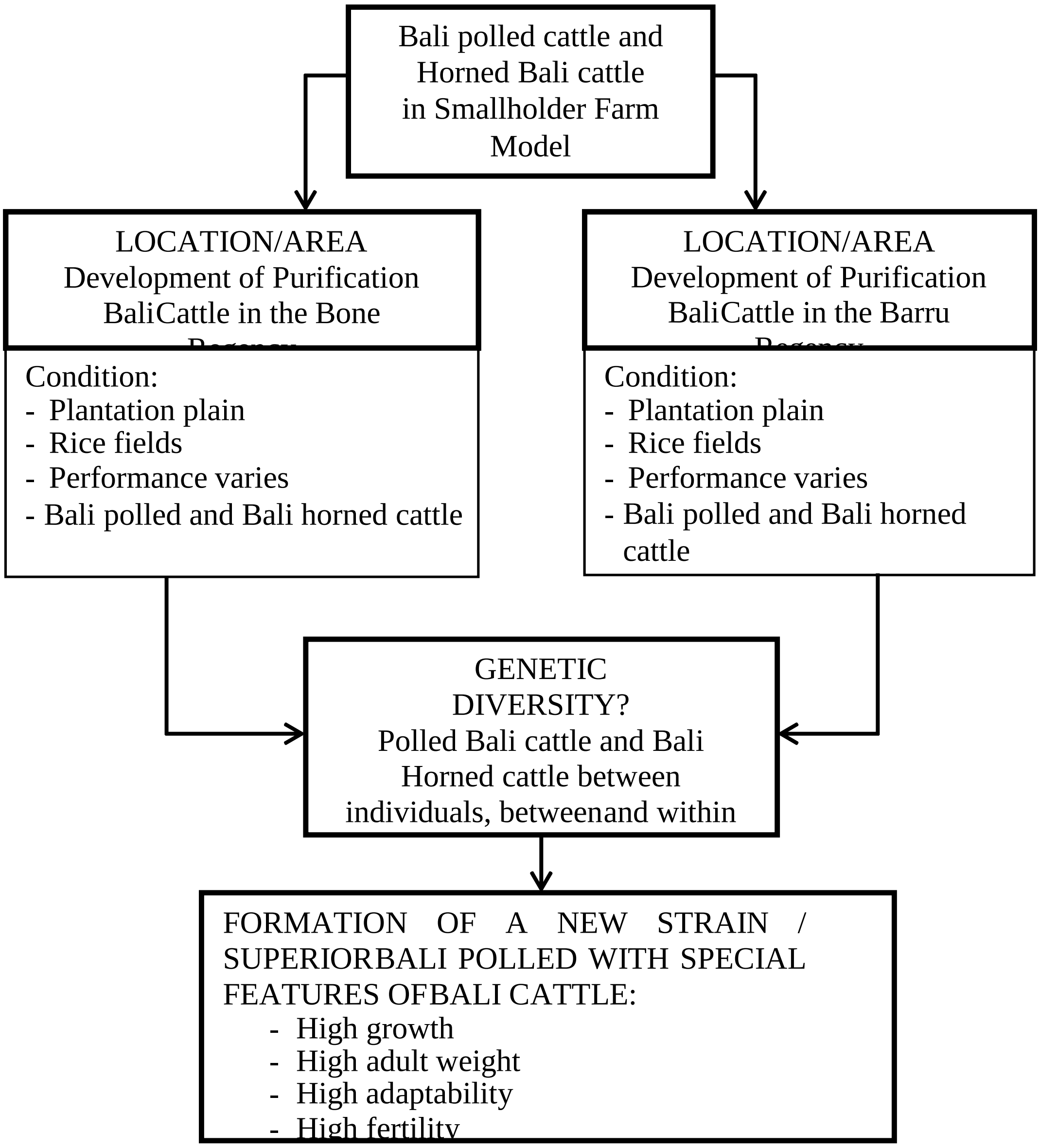


Figure 2. Scheme of Genetic Diversity Research Flow

Blood DNA extraction

DNA extraction was carried out to obtain genomic DNA. A total of 1 ml of blood was mixed with EDTA, transferred to a tube conical 10 ml, 4 ml lysis buffer, and 5 □l proteinase K 20 mg/ml, then shaken for 1 hour and then added phenol with ratio 1: 1, shaken for 30 minutes, and centrifuged at 2800 rpm for 15 minutes. The supernatant entered the conical, and then 1/10volume of 3 M Na acetate supernatant and 1:1 absolute ethanol were added, cooled at -80^{O}C for 1 hour, and centrifuged. Pellets obtained were washed with 70% ethanol and laminar dried

After drying, add 75 □l TE and store at 4^{O}C overnight. The DNA sample is ready to be used for further processing.

DNA amplification with PCR-RAPD

The primer used is six random primaries that will look for which one gives the best results amplification, composed of 10 bases. The PCR program starts with denaturation at an initial temperature of 95 ^{O} C for 4 minutes, a denaturation cycle at a temperature of 94 ^{O} C for 1 minute, *annealing at* a temperature of 35^{O}C for 1 minute, polymerization at72^{O}C for 2 minutes,polymerization finally at 72^{O}C for 10 minutes, next maintained at 4^{o}C for 5 minutes. Total cycles performed 45 times. Tube Stored in the freezer -20^{O}C until electrophoresis and documentation are completed.

Electrophoresis PCR product

The results of DNA amplification are separated based on heavy the molecule using 2 grams ofagarose gel in 100 ml of TAE buffer 1 x. Staining was carried out by adding 4 µl of ethidium bromide to the agarose gel, then pouring it into a mold that already had a comb. The comb is taken when the gel has hardened, then poured with 250 ml TAE 1 x. The DNA sample was provided with a solution of blue juice with a ratio of 5:2, and then the sample was entered intosuitable electrophoresis. Besides, It also has a marker installed as molecule standard. Electrophoresis runs at 50 volts for 60 minutes. DNA bands were observed under UV light andthen photographed with Polaroid film.

Data analysis

The bands from the electrophoresis results were analyzed statistically using the BSF method. The BSF calculation results are used to calculate differences or genetic diversity between the population of Bali cattle from the Bone district and district Barru. To calculate the BSF value in the population (between individuals within the population) it is calculated using the formula:

$$B_{ab} = 2b_{ab} / (ba + b_b)$$

Where ba and bb are the bands found in individuals a and b, bab is the number of bands in common between individuals a and b. BSF in the population (B) is the average B ab of possible pairs between individuals within the population [6]

The BSF value between individuals between populations (B') is calculated using the formula: $B' = 1 + B_{xy} – 0.5(B_x + B_y)$

B_x and B_y are the B values in populations x and y, and B_{xy} is the average band sharing betweenindividuals in populations x and y being compared [7]. Genetic differences and diversity werealso tested with SAS [8]. Genetic distance measurements $(\delta\mu)^2$ were used to measure genetic changes and were calculated by Microsat [9]. Based on the results of DNA fragment sequencing, a phylogenetic tree was created using the Computer software program MEGA 5 (Molecular Evolutionary Genetic Analysis) with the UPGMA (unweighted pair-group methodwith arithmetic).

RESULTS AND DISCUSSION

Phenotypic Characteristics of Polled Bali Cattle and Horned Bali Cattle

Results of observations of the characteristics of Bali cattle samples originating from twodifferent locations, namely Bone district and Barru district, show that Bali cattle have relatively uniform characteristics. The color of female Bali cattle is usually light brown, with athin black line running along the middle of the back. The color of the bull is brown when young,but then the color changes slightly darker at the age of 12-18 months, approaching black at maturity, except for castrated bulls that will retain their color chocolate. The characteristics of Bali cattle are generally uniform, namely brown in both females and males when young and close black when mature, white on the back of the thighs, the edge of the upper lip, the feathersat the tip of the tail are black, there is a clear black line on the part over the back [10]. This research used polled Bali and Bali cattle with horns, still calves under one-year-old. All livestock samples are the results of artificial insemination (AI) using polled Bali bull straw (Celebes and Kazep) and straw cow Bali horned (BIB Pucak belonging to the South Sulawesi Provincial Government).

Genetic Diversity of Bali Cattle Based on Molecular

This research analyzed PCR using several 10-base RAPD primers suitable for Bali cattle. In analyzing genetic diversity using RAPD, the level of consistency in the appearance ofa character under certain PCR conditions is very important, as the optimum reaction conditions for each type of organism may be different and must be determined first. Therefore, various PCR temperatures were also tested, apart from several primers.

The success of this technique is based more on the suitability of the primers, efficiency,and optimization of the PCR process [11]. Non-specific primers can cause other regions to be amplified in the genome that are not targeted or, vice versa, no genome regions are amplified. PCR optimization is also needed to produce the desired characters. This optimization concernsthe temperature of denaturation (separation of chains) and annealing (attachment of primer) ofDNA in a PCR machine. A low denaturation temperature can cause the double-stranded DNAnot to open, so new DNA polymerization is impossible. Attaching the primer to an open DNAstrand requires an optimum temperature because a temperature that is too high can cause amplification not to occur or vice versa; too low a temperature causes the primer to stick to theother side of the genome that is not the sidehomolog. As a result, many non-specific regions can be amplified in the genome.

Table 1 shows the results of amplification by using six RAPD primers on 51 samplesof Bali cattle originating from two different areas produced PCR products that could be readusing qualitative and quantitative analysis. From these results, there are bands with certain basepair sizes with specific patterns that do not always appear in all individuals or distinctivebands (Polymorphic bands), and bands that have a basepair size with *specific* patterns that always appear in each individual (Monomorphise bands).

Qualitative Analysis

The number and intensity of bands produced after DNA amplification by PCR-RAPD highly depend on the primer used. Primary selection in RAPD analysis influences the polymorphism of the resulting band because each primer has its binding sites. As a result, the polymorphic bands produced by each primer become different in size, number of base pairs, andamount of DNA bands.

The results of DNA amplification using six primers sometimes produced bands with different intensities. The purity and concentration of DNA greatly influence the intensity of theamplified bands on each primer. Specific bands are only found in individuals from certain populations with particular sizes. Table 1 shows the sequences of the six primers and the number of RAPD markers produced.

Table 1 shows the number of bands and polymorphic bands formed on the six primers. The banding pattern of DNA amplification results was obtained using different random primers.The results of PCR-RAPD amplification showed a banding pattern that needed to be more diverse, which can be seen from the total 61 bands, 68.9% of which were polymorphic bands in Bone Regency and 51.7% in Barru Regency. These results show that the genetic diversity of Bali cattle in the Bone Regency is still relatively high compared to the Barru Regency. The RAPD marker indicated this used had a high polymorphism level (68.9% vs 51.7% polymorphic bands). This may be because the Barru Regency is the center of Bali cattle breeding in South Sulawesi, so there is a possibility of higher inbreeding than in the Bone Regency. According to Hadiati and Mansyah [12], the difference in the number of polymorphicDNA fragments in the analysis of genetic diversity determines a population's diversity level. The difference in the number and polymorphism of DNA bands produced by each primer illustrates the complexity of the observed genome. Polymerase in cow Bali is also diverse, which can be seen by blood variations that reflect genetic differences.

It is said that the genetic diversity of Bali cattle in Bone Regency still has relatively high diversity due to the influence of genetic and environmental factors [13]. Opinion that observable diversity is caused by genetic diversity that arises from interactions between genetic and environmental factors. Genetic diversity is essential because of the factors that influence the response of a population to selection, both natural and artificial selections carried out by humanto exploit these biological resources according to their needs [14]. Populations with high levelsof genetic diversity have a better chance of survival. This is because each gene or combinationof genes has a different response to environmental conditions. The more diverse the genetic resources, the more resistant the population will be to survive in the long term and the higher the population's adaptability to increasingly more significant environmental changes. Genetic diversity is also essential in maintaining sustainability and increasing the productivity of a species.

Table 2 shows the differences in the number and intensity of bands in each primer. Thehighest percentage of polymorphic bands was in primer P04, with a percentage of 87.5%, and the lowest was in primer P10, with a rate of 11.1% in livestock samples in the Barru regency development area. This indicates that the DNA amplification results using the six primers aboveonly sometimes obtained bands with the same intensity. This difference was caused by the presence or absence of primer sequence pairs used in the DNA template. In primers P01 and P09, the number of bands produced is more significant than those made by other primers. Thebands obtained are the result of primer and DNA template amplification. The more sequences can be recognized by the primer on a DNA template, the more bands will be produced. The intensity of the amplified DNA band on each primer is greatly influenced by the purity and concentration of the DNA template containing compounds such as polysaccharides and phenolic compounds. Too small a concentration of DNA template often produces faint or unclear amplified DNA bands. Purwanta *et al,* [15] states that the DNA fragment patterns are determined by the presence or absence of primer attachment sites and by the purity and integrityof the DNA print. Impure template DNA prints/template will interfere with the attachment of the primer to its site and will inhibit the activity of the DNA polymerase enzyme. This enzymefunctions to polymerize DNA. Meanwhile, template DNA that experiences much fragmentation can eliminate the primer attachment site. This was clarified by the opinion of Prana and Hartati(2003), who stated that the success of genomic DNA amplification using the RAPD techniquewas determined not only by the sequence of primer bases used and their amount (primer contentin each reaction) but also by the suitability of PCR conditions including primer annealing temperature and extension.

Table 1 shows that the DNA bands from the six primers are 250 - 3000 base pairs, producing 61 polymorphic bands. The number of DNA bands was obtained from observationson 2% Agarose gel after electrophysics for 1 hour. This result was due to the range of DNA sizes that can be analyzed using the 2% agarose gene, which was around 100 - 2000 bp / 0.1 -

2.0 kb. The general range of DNA sizes that can be analyzed by 2% agarose gel is 0.1 - 2.0 kb[16].

Quantitative Analysis

A quantitative analysis was carried out by calculating the Band Sharing Frequency (BSF) values between individuals within the population and between populations in Bali cattlesamples in two Bali cattle development areas in South Sulawesi. Table 2 shows that the BSF value between individuals in the Bali polled cattle population both in the Bone Regency and Barru Regency ranges from 0.8008-0.9025 and 0.840-0.9043, respectively, while the horned Bali cattle BSF value ranges from 0.5745-0.7917 and 0.7929-0.9721, respectively. This showsthat the genetic diversity in both populations is still relatively high, although there are indications of inbreeding in Bali cattle in Barru Regency. However, with a good and continuousselection and breeding program, re-introducing the original variant of the Bali cattle bull everytwo years in the breeding program can control severe inbreeding.

The genetic diversity between individuals in the Bali cattle samples from a populationof Bone Regency or, in other words, the genetic similarity is relatively the same compared tothe Bali cattle samples population from Barru Regency, namely with BSF values of 1.0031 and 0.9846, respectively. This is because individuals in the Bali cattle population in both development areas have relatively the same genetic relationships. This is the opinion of Suwardi et al. [14], who stated that the factors that influence the formation of the population's genetic structure include factors that cause genes or increase genetic diversity, including mutation and immigration factors. At the same time, factors that reduce genetic diversity include natural selection and genetic drift.

Table 3 shows that the BSF value between individuals within and between populations is relatively large. This indicates that the genetic similarity between the two populations is quite significant, with an average BSF value with several primers approaching1,000. The high BSF value is due to excellent and continuous breeding or reintroducing of the original Bali cattle variant with superior offspring or inbreeding (Artificial Insemination)in Bali cattle in both Bali cattle development areas of South Sulawesi.

Table 1. Total Number of Polymorphic and Monomorphic Bands from Different RAPD Primers

Primers	Base Sequence	Total Bands			Polymorphic bands												Monomorphic bands												Basepairs Size
		Total	Bone	Barru	Bone						Barru						Bone						Barru						
					PP	%	TP	%	TT	%	PP	%	TP	%	TT	%	PP	%	TP	%	TT	%	PP	%	TP	%	TT	%	
P10	5`-GCG CGC ACT C-3`	10	10	9	5	50,0	4	40,0	8	80,0	4	44,4	4	44,4	1	11,1	5	50,0	5	50,0	1	10,0	4	44,4	5	55,6	8	88,9	250 - 1500
P06	5`-CTG CAG CCG T-3`	12	12	12	6	50,0	6	50,0	8	66,67	3	25,0	2	16,7	2	16,7	6	50,0	6	50,0	2	16,7	7	58,3	7	58,3	7	58,3	250 - 1300
P09	5`-CCA GGA CGC G-3`	12	12	12	8	66,7	5	41,7	7	58,33	6	50,0	10	83,3	9	75,0	4	33,3	7	58,3	3	25,0	6	50,0	2	16,7	3	25,0	250 - 2500
P01	5`-AGC TGT CTC A-3`	11	11	11	5	45,5	2	18,2	7	63,64	5	45,5	5	45,5	7	63,6	6	54,5	7	63,6	1	9,1	5	45,5	6	54,5	4	36,4	250 - 1000
P08	5`-CGC TTG GCG G-3`	8	8	8	5	62,5	2	25,0	5	62,5	2	25,0	3	37,5	3	37,5	3	37,5	6	75,0	3	37,5	4	50,0	4	50,0	4	50,0	500 - 3000
P04	5`-GGT CAC CTA C-3`	8	8	8	7	87,5	3	37,5	7	87,5	5	62,5	7	87,5	4	50,0	1	12,5	5	62,5	0	0,0	3	37,5	1	12,5	3	37,5	500 - 1500
Total		**61**	**61**	**60**	**36**	**59,0**	**22**	**36,1**	**42**	**68,85**	**25**	**41,7**	**31**	**51,7**	**26**	**43,3**	**25**	**41,0**	**36**	**59,0**	**10**	**16,4**	**29**	**48,3**	**25**	**41,7**	**29**	**48,3**	

PP = Bali polled cattle offspring of Bali polled bull TP = Horned Bali cattle offspring of Bali polled bull
TT = Horned Bali cattle offspring of horned Bali cattle bullSource: Research Data (2022)

Table 2. BSF Values between Individuals in the Population of Bali Cattle Samples in Boneand Barru Regencies in Each Primary

Primers	**Base Sequence**	**Bone District Area**		**Barru District Area**	
		Polled	**Hor ned**	**Polled**	**Horned**
P10	5’-GCG CGC ACT C-3’	0.8518	0.6424	0.8406	0.9721
P06	5’-CTG CAG CCG T-3’	0.8612	0.7698	0.9021	0.9047
P09	5’-CCA GGA CGC G-3’	0.8632	0.7530	0.8594	0.7929
P01	5’-AGC TGT CTC A-3’	0.9025	0.7049	0.8525	0.8690
P08	5’-CGC TTG GCG G-3’	0.8999	0.7917	0.9043	0.8796
P04	5’-GGT CAC CTA C-3’	0.8008	0.5745	0.7998	0.8381
Average		0.8632	0.7061	0.8598	0.8761

Source: Research Data (2022)

Table 3. BSF Values between Individuals within the Population and between Populations in BaliCattle Samples in Bone and Barru Regencies in Each Primary

Primers	**Base Sequence**	**BSF average values**		**Average**
		Bone	**Barru**	
P10	5’-GCG CGC ACT C-3’	1.0042	0.9587	0.9814
P06	5’-CTG CAG CCG T-3’	1.0107	1.0051	1.0079
P09	5’-CCA GGA CGC G-3’	0.9724	0.9933	0.9828
P01	5’-AGC TGT CTC A-3’	1.0181	1.0028	1.0104
P08	5’-CGC TTG GCG G-3’	0.9932	0.9907	0.9920
P04	5’-GGT CAC CTA C-3’	1.0203	0.9568	0.9885
Average		1.0031	0.9846	0.9939

Source: Research Data (2022)

Genetic Relationship and Distance

The genetic relationship of the polled Bali cattle population, horned Bali cattle descendantsof polled Bali bulls, and horned Bali cattle population descendants of horned Bali cattle in two Balicattle development areas in Bone and Barru districts can be seen in Table 4. The table shows the genetic relationship of polled and horned Bali cattle in both development locations with communityfarming (smallholders Farming) patterns ranging from 84 - 99%. This shows that Bali cattle developed by livestock communities in both areas still have a very close genetic relationship.

Table 4. Genetic Relationships between Polled Bali Cattle and Horned Bali Cattle in TwoDevelopment Areas in South Sulawesi

	PBr (1)	PTBr (2)	TTBr (3)	PBn (4)	PTBn (5)	TTBn (6)
PBr (1)		0.9501	0.9225	0.9043	0.9040	0.8679
PTBr (2)	0.0511		0.9334	0.9074	0.8999	0.8576
TTBr (3)	0.0807	0.0689		0.8564	0.8453	0.8264
PBn (4)	0.1005	0.0972	0.1550		0.9813	0.8795
PTBn (5)	0.1010	0.1055	0.1681	0.0189		0.8431
TTBn (6)	0.1416	0.1536	0.1907	0.1284	0.1707	

Genetic identity (upper diagonal) and genetic distance (lower diagonal); PBr : Bali polled cattle offspring of Bali polled bull in Barru Regency; PTBr : Horned Bali cattle offspring of Bali polled bull in Barru Regency;
TTBr : Horned Bali cattle offspring of horned Bali cattle bull in Barru Regency;PBn : Bali polled cattle offspring of Bali polled bull in Bone Regency;
PTBn : Horned Bali cattle offspring of Bali polled bull in Bone Regency;
TTBn : Horned Bali cattle offspring of horned Bali cattle bull in Bone Regency;

Table 4 also shows the genetic distance between the 5 % - 20 % of the Bali cattle populationin the two development areas and has a very close genetic identity (85 - 98%). This indicates crossbreeding or interbreeding between relatives in the Bali cattle population in the two Bali cattledevelopment areas. Furthermore, genetic diversity (genetic distance) was seen in the dendrogram image (Figure 3), showing that five identified clusters were present in the samples of the two Bali cattle development areas, polled and horned.

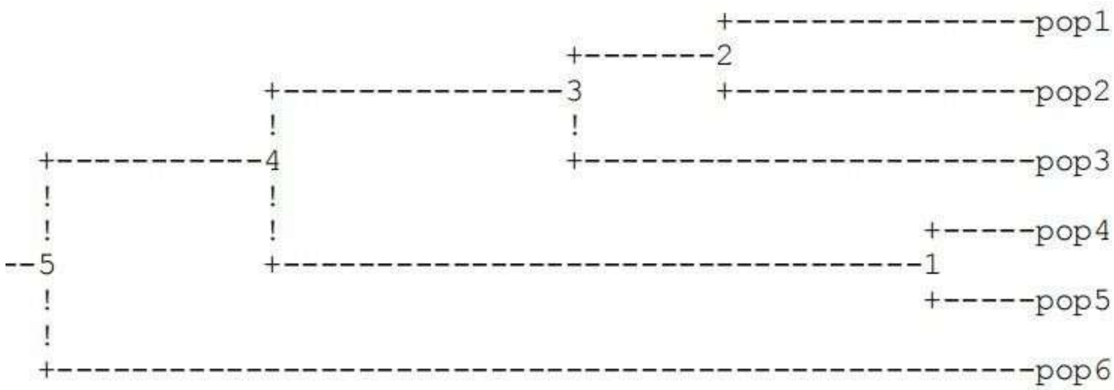


Figure 3. Genetic Relationship Dendogram with Several Different Primers

Description: Clusters 1 - 5, and pop1 - pop6

Between	And	Length
5	4	1.7902
4	3	2.3198
3	2	1.1837
2	Pop1	2.5570
2	Pop2	2.5570

Between	**And**	**Length**
3	Pop3	3.7408
4	1	5.1170
1	Pop4	0.9436
1	Pop5	0.9436
5	Pop6	7.8508

Pop1 : Bali polled cattle offspring of Bali polled bull in Barru Regency; Pop2 : Horned Bali cattle offspring of Bali polled bull in Barru Regency;

Pop3: Horned Bali cattle offspring of horned Bali cattle bull in Barru Regency;Pop4 : Bali polled cattle offspring of Bali polled bull in Bone Regency;
Pop5 : Horned Bali cattle offspring of Bali polled bull in Bone Regency;
Pop6 : Horned Bali cattle offspring of horned Bali cattle bull in Bone Regency;

The statistical analysis results of the NTSys program with the UPGMA method, it showsthat the fifty-one samples with six different primers were divided into 5 cluster groups and based ongenetic characters, the results depicted in the dendogram as showed in Figure 3, its show that thecoefficient of diversity of the relationship between populations is between 0.9436 - 7.85. This shows that the diversity of genetic kinship relationships at this level is still relatively high. The closest genetic distance is cluster 1 which highly closed pop5 and pop4. This is because the cattle in pop5and Pop4 are descendants of the first generation of Bali polled Bull in Bone district. While the furthest genetic distance is the cattle in Pop6 in cluster 5 with other cattle. This shows that the development of Bali polled cattle in Barru Regency has been carried out earlier than in Bone Regency, as indicated by the closer genetic distribution in the Barru area.

CONCLUSION

The BSF values between individuals in the population and the polled Bali cattle populationin the Bone and Barru districts fluctuated from 0.8008 to 0.9025 and 0.840 to 0.9043, respectively.In contrast, the horned Bali cattle BSF value ranged from 0.5745 to 0.7917 and 0.7929 to 0.9721. The genetic distance between the Bali cattle population was 5% to 20% in both livestock development areas. The results showed that the genetic diversity of polled Bali cattle was low compared to horned Bali cattle. The genetic relationship between polled and horned Bali cattle between the two development areas remained relatively close.

ACKNOWLEDGMENT

The authors express their most profound appreciation and gratitude for the assistance in carrying out this research, especially to Hasanuddin University through the Research and Community Service Institute, which has provided financial support in carrying out this research. Integrated Laboratory of the Faculty of Animal Husbandry, Hasanuddin University. We express ourgratitude to friends and colleagues who have helped with the success of this research and for all their assistance and support. Thanks very much.

REFERENCES

Entwistle, K. and D.R. Lindsay, "Strategies to Improve Bali Cattle in Eastern Indonesia". Proceedings of a Workshop 4–7 February 2002, Bali, Indonesia, Australian Centre for International Agricultural Research, Canberra. 2003.

N. Widyas, T. S. M. Widi, S. Prastowo, I. Sumantri, B. J. Hayes, and H. M. Burrow, “Promoting Sustainable Utilization and Genetic Improvement of Indonesian Local Beef Cattle Breeds: A Review,” *Agriculture*, vol. 12, no. 10, p. 1566, Sep. 2022, doi: 10.3390/agriculture12101566.

E. Kurnianto, “Improving genetic quality of local livestock for achieving food security,” *IOP Conf. Ser. Earth Environ. Sci.*, vol. 1041, no. 1, p. 012081, Jun. 2022, doi: 10.1088/1755-1315/1041/1/012081.

L. R. Ritz, M. Glowatzki-Mullis, D. E. MacHugh, and C. Gaillard, “Phylogenetic analysis ofthe tribe Bovini using microsatellites,” *Anim. Genet.*, vol. 31, no. 3, pp. 178–185, Jun. 2000,doi: 10.1046/j.1365-2052.2000.00621.x.

Baco, S., R. Malaka and L. Rahim, "Genetic Similarity between Bali Cattle and Crossbred with Simmental Breed". Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner,PP. 31 - 4, 2013.

M. Lynch, “The Similar Index and DNA Fingerprinting”, Mol. Biol. Evol., 7:478-487. 1990.

D. Maharani, “Genetic similarities within and between local quail and quail populations cruciferous based on analysis of polymerase chains reaction-random amplified polymorphic DNA (PCR-RAPD).,” *Livest. Bull.*, vol. 28, no. 4, pp. 184–193, 2004.

Baco, S., Zulkharnain, R. Malaka, Gozali R. Moekti. "Polled Bali Cattle and Potentials for the Development of Breeding Industry in Indonesia". Hasanuddin J. Anim. Sci . Vol.2, No. 1:23-33, 2020.

Z. Maupa, S. Baco, L. Rahim, and M. Yusuf, “Level of DNA similarity the horned and polledBali Cattle Using Microsatellite approach,” *Hasanuddin J. Anim. Sci.*, vol. 4, no. 2, pp. 125–136, Feb. 2023, doi: 10.20956/hajas.v4i2.21798.

R. Azis, V. M. A. Nurgiartiningsih, S. Wahjuningsih, H. Sudarwati, and A. Furqon, “Evaluation of qualitative characteristics of Bali cattle at Bali breeding center,” *E3S Web Conf.*, vol. 335, p. 00050, Jan. 2022, doi: 10.1051/e3sconf/202233500050.

M. Park, J. Won, B. Y. Choi, and C. J. Lee, “Optimization of primer sets and detection protocols for SARS-CoV-2 of coronavirus disease 2019 (COVID-19) using PCR and real- time PCR,” *Exp. Mol. Med.*, vol. 52, no. 6, pp. 963–977, Jun. 2020, doi: 10.1038/s12276- 020-0452-7.

R. P. Sri Hadiati, Ellina Mansyah, “Identifikasi molekuler beberapa varietas durian (Durio spp) menggunakan Random Amplified Polymorphic DNA (Molecular Identification of somedurian varieties using Random Amplified Polymorphic DNA),” . *Agrosains*, vol. 6, no. 2, pp.75 – 79., 2003.

Margawati, ET., SD. Volkandari, Indriawati and M. Ridwan, "Genetics Diversity and Relationships among Bali Cattle from Several Locations in Indonesia Based on ETH10 Microsatellite Markers". JITV, Vol. 23 No. 4: 168 – 173, 2018.

A. B. Suwardi, S. Syamsuardi, E. Mukhtar, and N. Nurainas, “The diversity and regional conservation status of wild edible fruit species in Sumatra, Indonesia,” *Biodiversitas J. Biol. Divers.*, vol. 24, no. 6, Jul. 2023, doi: 10.13057/biodiv/d240619.

Purwanta *et al.*, “Identifikasi Cacing Saluran Pencernaan (Gastrointestinal) Pada Sapi Bali Melalui Pemeriksaan Tinja Di Kabupaten Gowa,” *Agrisistem*, vol. 5, no. 1, pp. 10–21, 2009.

Muladno, "Dasar-Dasar Teknik DNA dan Beberapa Aplikasinya", Pusat Penelitian Biologi Bidang Zoologi, Lembaga Ilmu Pengetahuan Indonesia (LIPI), 2002.

RESPONSE OF ARTEMIA PARTHENOGENETICA FED ON DIFFERENT FEEDS

CHABET DIS Chalabia [1], FERHANI Khadra[1], BOUDJENAH Mustapha[1], MESLEM Nabila[2], DILMI Ammar[1], INCHACOUT Mohamed Djamal Eddine[1], ITCHIR Rachida[1], HEMEIR Abd el hafid[1].

1 Centre National de Recherche et de Développement de la Pêche et de l'Aquaculture CNRDPA, W. Tipaza,Algeria.

2 Ecole Nationale Supérieure des Sciences de la Mer et de l'Aménagement duLittoral, W. Alger, Algeria.

Corresponding author e-mail : ch.d.chalabia@gmail.com

ABSTRACT

Artemia is a necessary live food for a successful larval culture of many fish and crustaceans which has good adaptability with a broad food spectrum. Information on the response of *Artemia* to different feeds on a laboratory scale provides basic information, especially in enrichment cases. The present research was conducted at the CNRDPA Laboratory under standard conditions. *Artemia parthenogenetica* were fed on three different diets, Micro-algae with *Dunaliella sp*, the second treatment were fed on yeast, and the last on the *Ulva lactuca* powder. A statistical analysis was used to compare *Artemia* growth between various feed treatments. a significant difference in total length was observed between the three treatments for the juvenile stage and the adult stage where the yeast-fed nauplii became adults after only 15 days of culture with an average total length of

7.2 mm compared to the individuals from the 2nd batch (fed by the algal powder) with an average total length of

4.5 mm and individuals from the 3rd batch (fed by the micro-algae) with an average total length of 5.3 mm. The growth rate of *Artemia* individuals fed with yeast was more important for the juvenile and adult stages with values of 1.9 mm/Day and 0.3 mm/Day, respectively unlike the individuals fed with *Ulva* powder (0.3 mm/Day and 0.09 mm/Day for juveniles and adults, respectively) and individuals fed with micro-algae (0.7 mm/Day and

0.28 mm/Day for juveniles and adults, respectively). Regarding the survival rate, individuals fed with micro- algae have an important final survival rate (75%) compared to individuals fed with yeast (45%) and individuals fed with *Ulva* powder (20%). *Artemia* adopts a strategy of responding to different food conditions; it takes more time to reach an adult size for a high survival rate.

Keywords: *Dunaliella sp*, growth, Micro-algae, Survival rate, *Ulva lactuca,* yeast

INTRODUCTION

Artemia (Leach, 1819) (Branchiopoda, Anostraca) is the most abundant and common genus in hypersaline waters habitats worldwide, except in Antarctica (Van Stappen, 2002). *Artemia* belongs to Anostraca, the most ancient and primitive group among crustaceans, and has an advanced osmoregulation system among all animals, allowing them to exist in an extreme salinity habitat (Gajardo and Beardmore, 2012; Lantushenko et al., 2022).

Because Brine shrimp *Artemia* is the most used and important live feed in the crustacean, marine fish, and freshwater fish larvae-culture (Dhont and Sorgeloos 2002), the nutritional value, bio-ecological characterization, culture, and *Artemia* population dynamics have been relatively well studied in recent years (e.g., Amarouayache et al., 2009, 2012; Amarouayache and Kara, 2015; Chabet dis et al., 2021; Chabet dis et al.,2023), except under different feeding conditions.

In the face of the climate change scenario hitting the earth and affecting wetlands directly and indirectly through rising temperatures, changes in the intensity and frequency of rainfall, climatic events such as drought (Salimi et al., 2021), the culture of organisms that are essential on the market and for the development of aquaculture such as *Artemia*, proves to be essential. The main objective of this study is to evaluate the response of *Artemia* individuals to different feeds under standard conditions by studying growth and survival characteristics.

MATERIAL AND METHODS

The diameter of 100 hydrated cysts of *Artemia parthenogenetica* population from El Bahira Lake (Setif, Algeria)(in freshwater for 2 hours) and 100 decapsulated cysts after hydratation (immersion in sodium hypochlorite according to Lavens and Sorgeloos (1996)) was measured under a binocular microscope (Optika, Italy), equipped with a camera. The chorion thickness was calculated according to Vanhaecke and Sorgeloos (1980) according to the following formula:

chorion thickness =(diameter of hydrated cysts - diameter of decapsulated cysts)/2

Artemia parthenogenetica cysts from El Bahira Lake (Algeria) were hatched in seawater 35 PSU, at a temperature of 24 ºC and pH 8, and under continuous aeration and fluorescent lighting. After 24 hours of hatching, the nauplii were collected and cultured in standard laboratory conditions as described by Hontoria and Amat (1992).

For the hatching success, the hatching percentage and efficiency was calculated as:

Hatching percentage (%) = (Total number of cysts hatched/Total number of cysts)*100Hatching efficiency (nauplii/g) = Total number of nauplii/Mass of cysts (g)

The length of 30 nauplii was estimated under a binocular microscope (Optika, Italy), equipped with a

camera.

The study was conducted at 24 ± 1 °C under constant light in separate 2 L glass containers, with three feeding treatments (100% Micro-algae (*Dunaliella sp*), 100% brewer's yeast, and 100% *Ulva lactuca* powder) and three replications. *Ulva lactuca* powder was obtained after collection, cleaning, drying in the dark, and grinding. *Dunaliella sp* was cultured in standard conditions. The brewer's yeast was obtained from local market.

Survival was monitored by a direct count of each feeding treatments and of three replicates during the culture. Dead individuals were removed and the survival rate was calculated for each treatment.

For the growth experiments, the total lengths of 30 *Artemia* individuals were recorded using a binocularmicroscope (Optika, Italy) equipped with a camera, after anesthesia according to the method of Dana and Lenz (1986).

Absolute growth rate (AGR) was estimated as calculated by Wooton (1991): AGR = (final length-initial length)/culture days.

RESULTS

The average diameter of normal, hydrated and decapsulated cysts were 177.4 ± 14.1 µm, 235.26 ± 9.4 µm, 217.1

± 14.6 µm, respectively.

The hatching percentage of cysts of *Artemia parthenogenetica* from El Bahira Lake was 55.78% with a hatching efficiency of 28986.67 nauplii/g.

The average length of nauplii of *Artemia parthenogenetica* from El Bahira Lake was 377 ± 2.05 µm.

Survival rate decreased with increasing time culture. After 15 days, the highest survival was observed for *Artemia* individuals fed with micro-algae (75%), compared to *Artemia* individuals fed with brewer's yeast (45%) and *Artemia* individuals fed with Ulva powder (20%) (Fig.1).

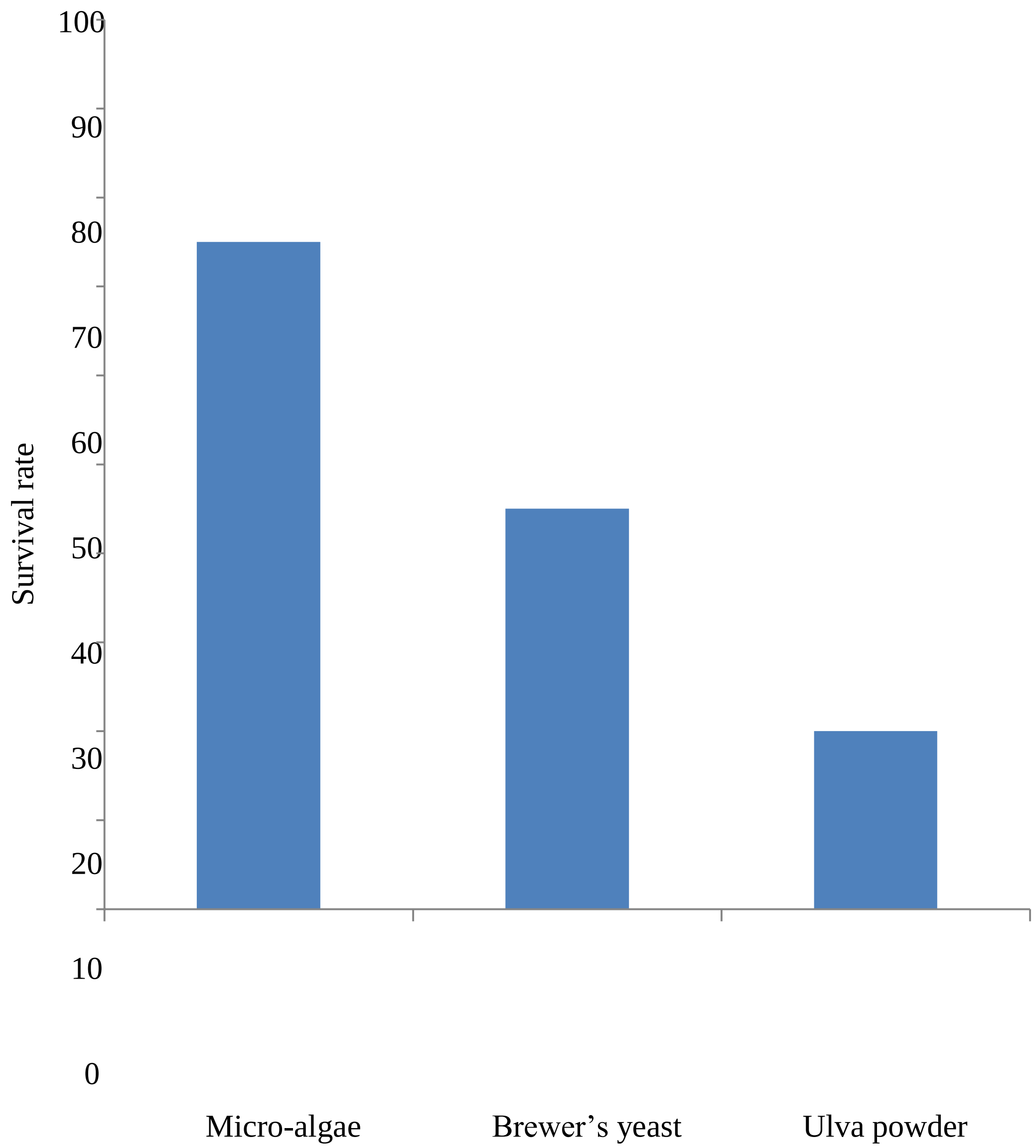


Figure 1. Percent survival of *Artemia parthenogenetica* from El Bahira fed on different feeds.

The growth experiments (total body length TL) for the three treatments are presented in Fig. 2,3,4.

At juvenile and adult, the average lengths were found to be significantly between the three treatments ($P<0.05$)

The nauplii fed on brewer’s yeast become adults after only 15 days of culture with an average total length of 7.2mm compared to the individuals from the 2nd treatment (fed by the algal powder) with an average total length of

4.5 mm and individuals from the 3rd treatment (fed by the micro-algae) with an average total length of 5.3 mm.

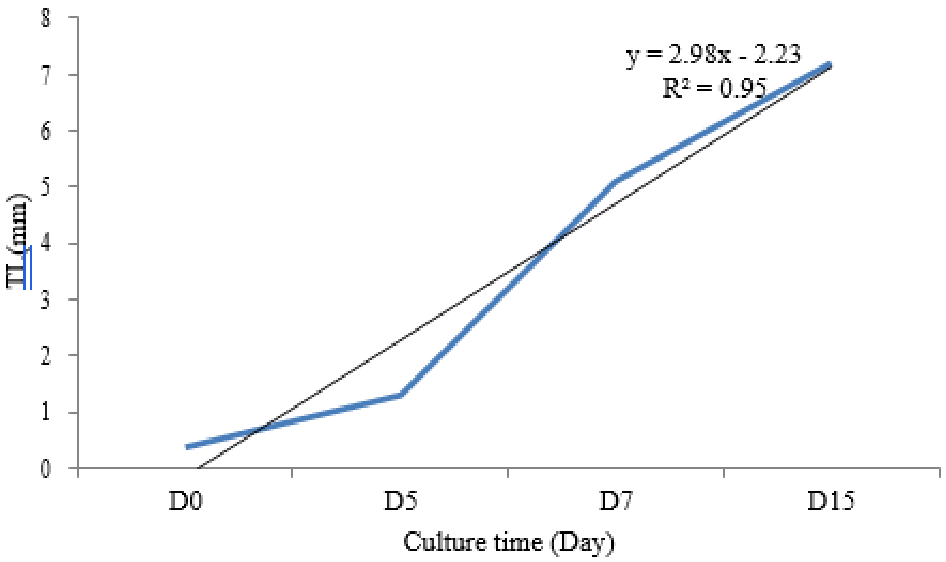


Figure 2. Growth of *Artemia parthenogenetica* from El Bahira fed on brewer's yeast.

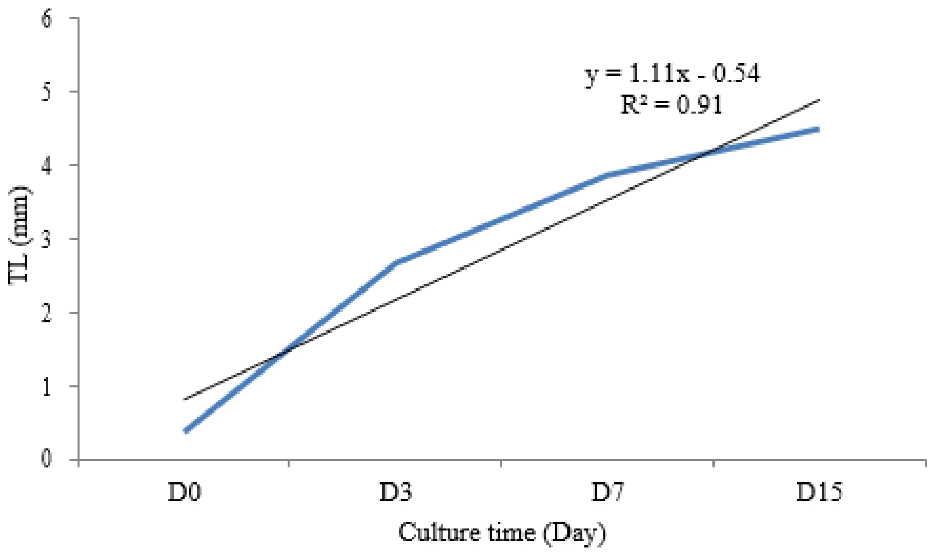


Figure 3. Growth of *Artemia parthenogenetica* from El Bahira fed on *Ulva lactuca* powder.

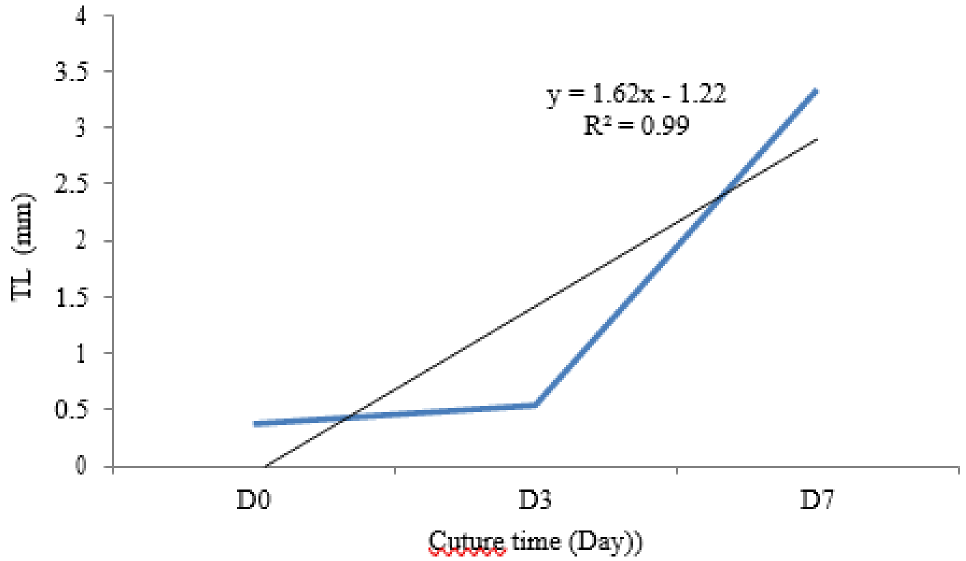


Figure 4. Growth of *Artemia parthenogenetica* from El Bahira fed on micro-algae.

The growth rate of *Artemia* individuals fed with yeast was more important for the juvenile and adult with values of 1.9 mm/Day and 0.3 mm/Day, respectively unlike the individuals fed with *Ulva* powder (0.3 mm/Day and

0.09 mm/Day for juveniles and adults, respectively) and individuals fed with micro-algae (0.7 mm/Day and 0.28mm/Day for juveniles and adults, respectively).

DISCUSSION

Artemia parthenogenetica from El Bahira Lake produced smaller cysts compared to the *Artemiaparthenogenetica* from Bethioua Sebkha (Chabet dis et al., 2021) located in western Algeria and *Artemia salina*

from Timimoune Sebkha (Chabet dis et al., 2021) located in southern Algeria. Vanhaecke and Sorgeloos (1980) reported that food and salinity variations in the biotope can affect cyst diameter and suggested that the diameter of the cysts was related to genetic characteristics.

The cyst hatching percentage of El Bahira is close to the hatching percentage of the El Melah (53.2%) and Bethioua (64.2%) populations in southern and western of Algeria, respectively. (Chabet dis et al., 2021). The result of the hatching efficiency for El Bahira cysts is lower than the values reported by Chabet dis et al. (2021) for the populations of El Melah (southern Algeria), Timimoune (southern Algeria) and Bethioua (western Algeria). Compared with other cysts, the hatching percentage obtained in the present study can be considered average and acceptable compared to the values reported by several authors between 20% and 90% hatching (Sorgeloos et al., 1986). However, Chabet dis et al. (2021) reported that hatching quality can be improved by decapsulation of cysts. Therefore, harvesting at the time of cyst production is important to ensure better hatching quality.

Also, *Artemia* from El Bahira Lake produced smaller nauplii than other Algerian strains (Bethioua (478.73 ± 0.2 µm), Timimoune (453.26 ± 0.3 µm) reported by chabet dis et al. (2021)). Chabet dis et al. (2021) reported that Naupliar size is important in view of the potential use as live food in aquaculture.

Percent survival and growth of *Artemia* from El Bahira Lake varied depending on each food used. *Artemia* is known to be filter feeder zooplankton nonselective (Dhont and Sorgeloos, 2002), based on diet size ranging between 1µm and 50µ and optimal about 16 µm (Fernández, 2001). Among the foods reported in *Artemia*, we find yeast (Dhont and Sorgeloos, 1996; Lavens and Sorgeloos, 1991). Amarouayache et al. (2007) fed *Artemia* from Sebkha Ez-Zemoul with yeast and reported a growth rate of 0.33 ± 0.3 mm/Day with a longer juvenile stage, about 10 Days where metanauplii grow from a total length of 1.73 mm to 5.11 mm to become pre-adults. However, micro-algae are the natural food of *Artemia* (Mohebbi, 2016). Micro-algae are used as standard feed to standardize culture and reproduction conditions (Chabet dis et al., 2023) even though it is expensive (Sulistiyarto and Bakrie, 2024). Other sources have been reported to be food for *Artemia* such as bacteria, waste products from the food industry and dried algae (Dhont and Sorgeloos, 1996; Lavens and Sorgeloos, 1991). Coming out obtained results, *Artemia* displayed an adaptive strategy to food category based on time and energy costs.

REFERENCES

Amarouayache, M., Derbal, F., & Kara, M. H. (2007). Croissance et reproduction d'*Artemia* sp. de la Sebkha Ez-Zemoul (Algérie Nord-Est) dans les conditions d'élevage. 38ème Congrès de la CIESM, Istanbul (Turquie), 9- 13 Avril 2007

Amarouayache, M., Derbal, F., & Kara, M. H. (2009). Biological data on *Artemia salina* (Branchiopoda, Anostraca) from Chott Marouane (northeast Algeria). *Crustaceana*, 997-1005. *Crustaceana* 82: 997–1005.https://doi.org/10.1163/156854009X452768.

Amarouayache, M., Derbal, F., & Kara, M. H. (2012). Note on the carcinological fauna associated with *Artemia salina* (Branchiopoda: Anostraca) from Sebkha Ez-moul (northeast Algeria). *Crustaceana* 85, 129–137. https://doi.org/10.1163/156854012X623728.

Amarouayache, M., & Kara, M. H. (2015). Quality evaluation of a new strain of *Artemia* from Sebkha Ez-moul, Algeria: biometry, hatching and fatty acid composition. *Vie et Milieu* 65,211–217.

Chabet dis, C., Refes, W., Varo, I., Hontoria, F., Amat, F., & Navarro, J. C. (2021). Quality evaluation of *Artemia* cysts from three Algerian populations. *African Journal of Aquatic Science*, *46*(4), 464-472. https://doi.org/10.2989/16085914.2021.1895052

Chabet dis, C., Refes, W., & Okazaki, R. K. (2023). First biocharacterization of *Artemia* populations from western and northwestern Algeria. *Zoomorphology*, *143*(1), 77-88. https://doi.org/10.1007/s00435-023-00626-3

Dana, G. L., & Lenz, P. H. (1986). Effects of increasing salinity on an *Artemia* population from Mono Lake, California. *Oecologia*, *68*, 428-436. https:// doi. org/ 10. 1007/ BF010 36751

Dhont, J., & Sorgeloos, P. (1996). Tank production and use of on grown *Artemia*. In: Lavens P, Sorgeloos P, editors. Manual on the production and use of live food for aquaculture. Rome: *FAO Fisheries Technical Paper*, 361, 164-195.

Dhont, J., & Sorgeloos, P. (2002). Application of *Artemia*. In: Abatzopoulos TJ, Beardmore JA, Clegg JS (Eds), *Artemia*: *Basic and applied biology*. Dordrecht: Kluwer Academic Publishers. pp 251–277. https://doi.org/10.1007/978-94-017-0791-6_6.

Fernández, R. G. (2001). *Artemia* bioencapsulation I. Effect of particle sizes on the filtering behavior of *Artemia franciscana*. *Journal of Crustacean Biology*, *21*(2), 435-442.

Gajardo, G. M., & Beardmore, J. A. (2012). The brine shrimp *Artemia*: adapted to critical life conditions.*Frontiers in physiology*, *3*, 185.

Hontoria, F., & Amat, F. (1992). Morphological characterization of adult *Artemia* (Crustacea, Branchiopoda) from different geographical origin. Mediterranean populations. *Journal of plankton research*, *14*(7), 949- 959.https:// doi. org/ 10. 1093/ plankt/ 14.7. 949

Lantushenko, A., Meger, Y., Gadzhi, A., Anufriieva, E., & Shadrin, N. (2022). *Artemia spp*.(Crustacea, Anostraca) in crimea: new molecular genetic results and new questions without answers. *Water*, *14*(17), 2617. https://doi.org/10.3390/w14172617

Lavens, P., & Sorgeloos, P. (1991). Production of *Artemia* in culture tank. In: Browne RA, Sorgeloos P, TrotmanCNA, editors. *Artemia* Biology. *CRC*, 317-350.

Lavens, P., & Sorgeloos, P. (1996). Manual of the production and use of live food for aquaculture. *FAO Fisheries Technical Paper* 361.

Mohebbi, F., Hafezieh, M., Seidgar, M., Hosseinzadeh Sahhafi, H., Mohsenpour Azari, A., & Ahmadi, R.(2016). The growth, survival rate and reproductive characteristics of *Artemia urmiana fed* by *Dunaliella tertiolecta*, *Tetraselmis suecica*, *Nannochloropsis oculata*, *Chaetoceros sp*., *Chlorella sp*. and *Spirolina sp*. as feeding microalgae. *Iranian Journal of Fisheries Sciences*, 15, 727-737.

Salimi, S., Almuktar, S. A., & Scholz, M. (2021). Impact of climate change on wetland ecosystems: A critical review of experimental wetlands. *Journal of Environmental Management*, *286*, 112160.

Sorgeloos, P., Lavens, P., Leger, P., Tackaert, W., & Versichele, D. (1986). Manual for the culture and use of brine shrimp *Artemia* in aquaculture. Belgium: *Artemia* Reference Center, University of Ghent.

Sulistiyarto, B., & Bakrie, R. (2024). Survival, growth, and biomass of brine shrimp (*Artemia franciscana*) fed with *Spirulina* powder and soybean flour. *International Journal of Fisheries and Aquatic Studies*, 12, 13-18.

Van Stappen, G. (2002). Zoogeography. In : *Artemia* Basic and Applied Biology. T.J. Abatzopoulos, J.A. Beardmore, J.S. Clegg, P. Sorgeloos (eds), Kluwer Academic Pub. (pp. 171-224). Dordrecht, The Netherlands, pp : 171-224

Vanhaecke, P., & Sorgeloos, P. (1980). International Study on *Artemia*. IV. The biometrics of *Artemia* strains from different geographical origin. *The brine shrimp Artemia*, *3*, 393-405.

Wooton, RJ. (1991) Ecology of teleost fishes: fish and fisheries. Springer, Heidelberg, The Netherland

COMPARISON OF ORIGINAL EQUIPMENT MARKET FILTER AND UNORIGINAL AFTERMARKET FILTER PERFORMANCES FOR THE DRINKING WATER PURIFICATION SYSTEMS: FROM THE PERSPECTIVE OF ACCUMULATIONS OF POTENTIALLY TOXIC ELEMENTS

İpek Atılgan HELVACIOĞLU[1]*, *Hayati Arda*[2], *Cem TOKATLI*[1]

[1]*Trakya University, Evrenos Gazi Campus, İpsala Vocational School, Department of Laboratory Technology, Edirne / Türkiye*

[2]*Trakya University, Faculty of Science, Department of Biology, Edirne / Türkiye*

**Corresponding Author:* ipekhelvacioglu@trakya.edu.tr

ABSTRACT

Household Drinking Water Purification Systems (WPS) enable us to produce healthy and high-quality water in our homes. However, in this application, it is very important to maintain our device and change its filters periodically. Original equipment market (OEM) filters are those that are manufactured and sold by the same manufacturer as the original equipment piece. On the other hand, unoriginal aftermarket (UAM) filters are those that have not been made by the same manufacturer but have been fashioned to fit as well as the original parts. In this study, the performances of OEM filter and UAM filter for one of the most popular WPS in Türkiye were evaluated. Unpurified and purified tap water samples were taken from İstanbul Province in winter season of 2021. Four significant potentially toxic elements including nickel (Ni), arsenic (As), cadmium (Cd) and lead (Pb) were measured in drinking water samples and how much the WPS with OEM and UAM filters improves these parameters were determined. As a result of this research, it has been clearly demonstrated that the OEM filtered WPS significantly improved the drinking water quality by decreasing the total elemental content approximately 50%, while the UAM filtered WPS did not improve the water quality, and even increased the levels of many pollutant parameters in the water (total elemental content increased by approximately 30%).

Keywords: Water purification systems, OEM – UAM filters, Potentially Toxic Elements

INTRODUCTION

The history of water purification is indeed highlighting the evolution of the technology over time. Water is known as purified by using natural materials like stones, sand, and by boiling at the ancient times. Modern water treatment milestones may be summarised as: in 1903, the first water softening device was developed; in 1980, the first membrane for water purification devices was developed; in 1995, UV technology was integrated to the purification devices; in 2001, the first closed water purification system was developed; in 2007, carbon filters that provide the mineral support in water purification devices were developed; in 2015, more portable water purification systems were developed. These advancements reflect the continuous efforts to improve water quality and accessibility. Each milestone represents a significant leap in technology, making water purification more efficient and accessible (Maden et al., 2019; Tokatlı and Ustaoğlu, 2021).

Toxic elements have numbers of hazardous effects both on the ecological balance of environment and on the human health. They may cause various non-carcinogenic and carcinogenic health problems and diseases (Tokatlı, 2015; 2017; 2019; 2021; Wagh et al., 2018; Xiao et al., 2019; Tokatlı and Ustaoğlu, 2020; Köse et al., 2020; Tokatlı et al., 2021; Varol and Tokatlı, 2021; Tokatlı and Varol, 2021).

The healthy operation of household WPSs, which are found in many people's homes today, depends on their periodic maintenance being meticulously carried out and their filters being changed regularly. In addition to the original equipment market (OEM) filters provided by the manufacturers, many unoriginal aftermarket (UAM) filters that have not been made by the same manufacturer are also quite common in the market. In the current investigation, the performances of OEM and UAM filters for one of the most popular WPS in Türkiye were evaluated by measuring four significant potentially toxic elements (Ni, As, Cd and Pb) in filtered and unfiltered drinking water samples.

MATERIALS AND METHODS

Collection of Water Samples

Drinking water samples were taken from the tap water of İstanbul Province (Küçükçekmece District), where is known as the most populated city in Türkiye, and from the purified tap water treated by OEM and UEM filters by a widely used household water purification system with reverse osmosis in the winter season of 2021. Water samples were taken to the 1 L pre-cleaned and acid washed polyethylene bottles. pH of water samples to be used in the elemental analyses were reduced with nitric acid in order to make them below 2 (APHA, 2005).

Water Purification Stages of Investigated Household WPS

5 different filtering systems are used in the investigated WPS. Sediment Pre-Filter (SPF) (1) collects coarse dirt. Granular Activated Carbon Filter (GACF) (2) retains chlorine and other gases and gives clarity to water. Block Carbon Filter (BCF) (3) is a second carbon filter in addition to the GACF filter. It makes the incoming water to enter the membrane. It holds even the finest particles. Membrane (M) (4) is the heart of the reverse osmosis system. It separates all negative elements in water except water molecules. The Final Carbon Filter (FCF) (5) gives flavour to the water and removes the odour that may occur in the tank (http://www.cebilon.com.tr/).

Potentially Toxic Element Analysis

Water samples were filtered and their volumes have been set to 50 ml with ultra – pure water. Then the macro – micro element contents were measured by an Agilent branded (7700 XX) ICP-MS in the central laboratory of Thrace University (accreditation certificated laboratory). All the macro – micro element analyses were listed as the average of triple reads (TS EN / ISO IEC 17025) (EPA, 2001).

RESULTS AND DISCUSSION

The detected potentially toxic element levels in tap and purified water samples by OEM and UEM filters and the percent exchanges between the tap and purified water by OEM and UEM filters are given in Table 1 and Figure 1.

Arsenic, nickel, cadmium and lead are known as carcinogenic, hazardous and quite toxic elements. Exposure of these toxicants may cause many significant health problems (Çiçek et al., 2013; Köse et al., 2015; Bhowmick et al., 2018; Ustaoğlu et al., 2022; Din et al., 2023; Haque et al., 2023; Mia et al., 2023; Mutlu et al., 2023; Yüksel et al., 2024). In this study, the highest improvement rates were determined in the OEM filters for arsenic and nickel parameters, which were recorded as 64% and 58%, respectively. It was also determined that the UEM filters reduced the arsenic parameter by only 10%, while it was determined that the nickel levels increased in purified drinking water by the UEM filters by approximately 96% instead of decreasing. In addition, the OEM filters reduced the lead and cadmium levels by 36% and 15%, respectively, while the UEM filters increased these element levels by 27% for lead and 18% for cadmium.

Table 1. Detected data (ppm) and percentage exchanges

	İstanbul Tap Water	**Purified by UEM Filter**	**Percentage Exchange***
Ni	3.880	7.595	+95.75
As	0.273	0.246	-9.83
Cd	0.067	0.079	+18.48
Pb	0.656	0.833	+26.96
	İstanbul Tap Water	**Purified by OEM Filter**	**Percentage Exchange***
Ni	5.133	2.145	**-58.21**
As	0.223	0.080	**-63.89**
Cd	0.053	0.045	-15.23
Pb	0.940	0.599	**-36.33**

*Reduces after purification more than 30% are marked in bold

CONCLUSIONS

In this study, the performances of the OEM and UEM filters in a widely used water purification system (WPS) in Türkiye were evaluated. According to the results of this research, it has been clearly documented that the OEM filtered WPS significantly improved the drinking water quality by decreasing the total elemental content approximately 50%, while the UAM filtered WPS did not improve the water quality, and even increased the levels of many pollutant parameters in the water (total elemental content increased by approximately 30%). Therefore, in line with our research data, it is recommended to use original products provided by the manufacturer in the household water purification systems.

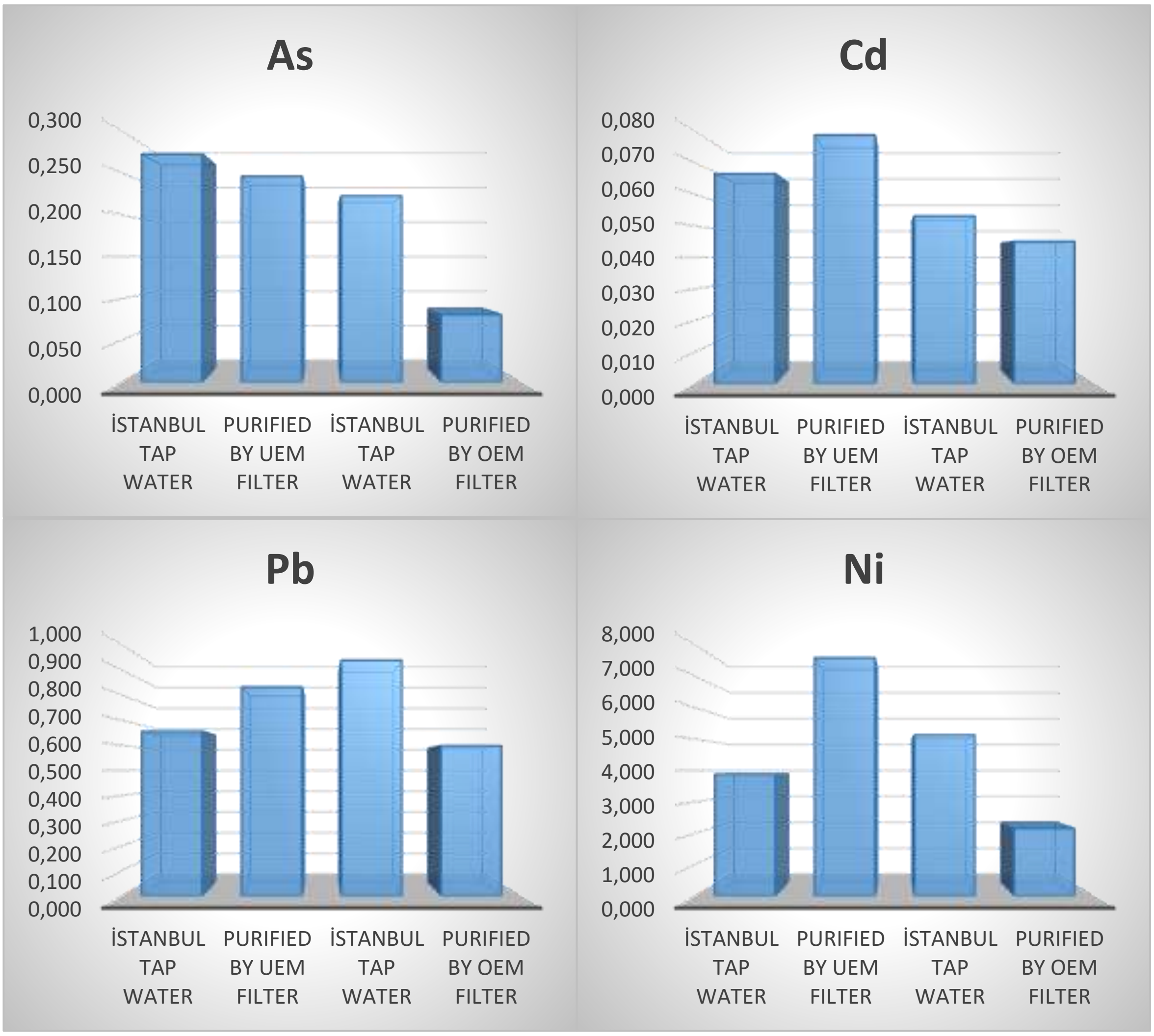


Figure 1. Comparison of purification performances (ppm)

REFERENCES

APHA, 2005. Standard Methods for the Examination of Water and Waste Water. American Public Health Association, Washington DC.

Bhowmick, S., Pramanik, S., Singh, P., Mondal, P., Chatterjee, D., Nriagu, J., 2018. Arsenic in Groundwater of West Bengal, India: A Review of Human Health Risks and Assessment of Possible Intervention Options. Science of the Total Environment, 612: 148–169.

Çiçek, A., Bakış, R., Uğurluoğlu, A., Köse, E., Tokatlı, C., 2013. The Effects of Large Borate Deposits on Groundwater Quality. Polish Journal of Environmental Studies, 22 (4): 1031-1037.

Din, I. U., Muhammad, S., Rehman, I., Tokatlı, C., 2023. Spatial Distribution of Potentially Toxic Elements Contamination and Risk Indices of Water and Sediments in the Darband and Samana Streams, Pakistan. Environmental Monitoring and Assessment, 195: 1343.

Environmental Protection Agency (EPA) METHOD 200.7., 2001. Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry.

Haque, J., Siddique, A. B., İslam, S., Ali, M. M., Tokatlı, C., İslam, A., Pal, S. C., İdris, A. M., Malafaia, G., İslam, A. R. T., 2023. Effects of Covid-19 Era on a Subtropical River Basin in Bangladesh: Heavy Metal(Loid)s Distribution, Sources and Probable Human Health Risks. Science of the Total Environment, 857: 159383.

http://www.cebilon.com.tr/

Köse, E., Çiçek, A., Uysal, K., Tokatlı, C., Emiroğlu, Ö., Arslan, N., 2015. Heavy Metal Accumulations in Water, Sediment and Some Cyprinidae Fish Species from Porsuk Stream (Turkey). Water Environment Research, 87 (3): 195-204.

Köse, E., Emiroğlu, Ö., Çiçek, A., Aksu, S., Başkurt, S., Tokatlı, C., Şahin, M., Uğurluoğlu, A., 2020. Assessment of Ecologic Quality in terms of Heavy Metal Concentrations in Sediment and Fish on Sakarya River and Dam Lakes, Turkey. Soil and Sediment Contamination: An International Journal, 29:3, 292-303.

Maden, H., Çetinkaya, K., Evlen, H., 2019. Historical Development of Water Treatment Equipment and Optimization According to Sink Dimensions. Gazi Journal of Engineering Sciences, 5 (1): 77-90.

Mia, Y., İslam, A. R. T., Jannat, J. N., Jion, M. M. F., Sarker, A., Tokatlı, C., Siddique, A. B., İbrahim, S. M., Senapathi, V., 2023. Identifying factors affecting irrigation metrics in the Haor Basin using integrated Shannon's Entropy, fuzzy logic and automatic linear model. Environmental Research, 226: 115688.

Mutlu, E., Tokatlı, C., Islam, A. R. T., İslam, S., Muhammad, S., 2023. Water quality assessment of Şehriban Stream (Kastamonu, Türkiye) from a multi-statistical perspective. International Journal of Environmental Analytical Chemistry, https://doi.org/10.1080/03067319.2023.2197114.

Tokatlı, C., 2015. Assessment of the Water Quality in the Meriç River: As an Element of the Ecosystem in the Thrace Region of Turkey. Polish Journal of Environmental Studies, 24 (5), 2205-2211.

Tokatlı, C., 2017. Bio – Ecological and Statistical Risk Assessment of Toxic Metals in Sediments of a Worldwide Important Wetland: Gala Lake National Park (Turkey). Archives of Environmental Protection, 43 (1): 34-47.

Tokatlı, C., 2019. Drinking Water Quality Assessment of Ergene River Basin (Turkey) by Water Quality Index: Essential and Toxic Elements. Sains Malaysiana, 48 (10): 2071-2081.

Tokatlı, C., 2021. Health Risk Assessment of Toxic Metals in Surface and Groundwater Resources of a Significant Agriculture and Industry Zone in Turkey. Environmental Earth Science, 80: 156.

Tokatlı, C., Ustaoğlu, F., 2020. Health Risk Assessment of Toxicants in Meriç River Delta Wetland, Thrace Region, Turkey. Environmental Earth Science, 79: 426.

Tokatlı, C., Ustaoğlu, F., 2021. Evaluation of a Household Drinking Water Purification System Performance in Terms of Organic – Inorganic Water Pollution Indicators and Ecological – Health Risk Assessment Indices. International Journal of Agriculture Environment and Food Sciences, 5 (3): 355-363.

Tokatlı, C., Uğurluoğlu, A., Köse, E., Çiçek, A., Arslan, N., Dayıoğlu, H., Emiroğlu, Ö., 2021. Ecological Risk Assessment of Toxic Metal Contamination in a Significant Mining Basin in Turkey. Environmental Earth Science, 80 (17): 1-19.

Tokatlı, C., Varol, M., 2021. Variations, Health Risks, Pollution Status and Possible Sources of Dissolved Toxic Metal(loid)s in Stagnant Water Bodies Located in an Intensive Agricultural Region of Turkey. Environmental Research, 201: 111571.

Ustaoğlu, F., İslam, S., Tokatlı, C., 2022. Ecological and Probabilistic Human Health Hazard Assessment of Heavy Metal in Sera Lake Nature Park Sediments (Trabzon, Turkey). Arabian Journal of Geosciences, 15: 597.

Varol, M., Tokatlı, C., 2021. Impact of Paddy Fields on Water Quality of Gala Lake (Turkey): An Important Migratory Bird Stopover Habitat. Environmental Pollution, 287: 117640.

Wagh, V. M., Panaskar, D. B., Mukate, S. V., Gaikwad, S. K., Muley, A. A., Varade, A. M., 2018. Health Risk Assessment of Heavy Metal Contamination in Groundwater of Kadava River Basin, Nashik, India. Modelling Earth Systems and Environment, 4: 969–980.

Xiao, J., Wang, L., Deng, L., Jin, Z., 2019. Characteristics, Sources, Water Quality and Health Risk Assessment of Trace Elements in River Water and Well Water in the Chinese Loess Plateau. Science of the Total Environment, 650: 2004-2012.

Yüksel, B., Ustaoglu, F., Aydın, H., Tokatlı, C., Topaldemir, H., Islam, S., Muhammad, S., 2024. Appraisal of Metallic Accumulation in the Surface Sediment of a Fish Breeding Dam in Türkiye: A Stochastical Approach to Ecotoxicological Risk Assessment. Marine Pollution Bulletin, 203: 116488.

EVALUATIONS OF THE BIOLOGICAL ASPECTS OF THE EUROPEAN EEL (ANGUILLA ANGUILLA) LOCAL STOCK AND THE SILVERING PROCESS, IN THE KARAVASTA LAGOON, ALBANIA.

MARCO KULE[1], EDMOND HALA[2], ELVIS KAMBERI[2].

[1.] *Ministry of Agriculture and Rural Development, Directory of Aquaculture and Fisheries Services.*
[2.] *Agricultural University of Tirana, Faculty of Agriculture and Environment, Department of Animal Production, Kodër Kamëz, Tirana, Albania.*

** Corresponding author: marcokule11@gmail.com*

ABSTRACT

The European eel, *Anguilla anguilla*, is currently facing a critical endangerment primarily due to fishing activities and the numerous obstacles it encounters on its journey to its breeding area. Initiated in 2022, this research is a component of a thorough three-year study and serves as the primary exploration of the European eel population in the South-Eastern area of the Adriatic Sea. The present research was conducted in the Karavasta lagoon, in the Albanian territory, where a total of 140 eel specimens were collected by the end of December 2023 using a fyke-net barrier placed both upstream and downstream of the fish barrier, a common method used to capture this species in Albanian lagoons. This phase of the study focuses on biological aspects such as the distribution of length and weight frequencies, as well as the sex ratio of the eels. Furthermore, parameters like eye diameter, pectoral fin length, and Hartmann's index were analyzed to determine the maturity stage of the eels. At the same time, the health status of migratory silver eels was assessed, with special attention paid to parasitic infestations with the nematode *Anguillicola crassus*. The results from this part of the research indicate a negative allometric growth trend in the samples. It is worth noting that the eels did not display any signs of parasitic infestions, as none of the 140 individuals were found to be infested with the nematode *Anguillicola crassus*. As the study progresses to include additional parameters for future examination, it is expected that valuable insights will be obtained regarding this specific area of the Adriatic Sea, which has not been thoroughly investigated in relation to the European eel.

Keywords: *European eel**;** length frequency; stock; growth.*

INTRODUCTION

The European eel (Anguilla anguilla, Linnaeus 1758) is classified as a catadromous species, origination in the North Atlantic. Through an extensive migratory journey and a series of metamorphic changes from leptocephalus to glass eels, they eventually inhabit regions across Europe, North Africa, and the Mediterranean Sea [1]. During this phase, they occupy freshwater, brackish, and coastal environments, undergoing a transformation into yellow eels. Upon reaching sexual maturity as silver eels, they undertake a singular migration back to the Sargasso Sea to spawn, after which they die [1, 2].

The European eel is a species of particular concern due to the dramatic decline in its population over the past decades [3]. Since the 1970s the population of European eels has experienced a staggering decline of 99%. This significant reduction can be attributed to unsustainable fishing practices and various anthropogenic factors. Longitudinal studies of eel species (Anguilla spp.) over the past forty years reveal a substantial global decrease in their populations. *Anguilla anguilla* has already been placed on the IUCN Red List of critically endangered species [4]. Following the 1970s, the European eel, which is the most commercially significant species, has faced a 99% reduction in its population [1]. Contributing factors to this decline include overfishing, climate change, decreased recruitment of glass eels, obstacles to migration in river systems, habitat degradation, increased parasitism, pollution, the re-oligotrophization of freshwater ecosystems, a decline in available prey, and predation by cormorants *(Phalacrocorax spp.)*[5,6]. The alarming status of eel populations in Europe has prompted the implementation of recovery measures in accordance with European Council Regulation 1100/2007 [7], alongside management strategies for eel fisheries (http://ec. europa. eu/fisheries/marine_species/wild_species/eel/ management_plans/) [8].
Albania has a long history of utilizing eel for culinary purposes, yet comprehensive research on this species within its territory remains lacking. The current study seeks to investigate the morphometric relationships between length and weight (LWR), marking the inaugural research on this species in one of Albania's eight lagoons, specifically the Karavasta lagoon. The LWR serves as a valuable metric for evaluating the health of silver eel individuals as they prepare for their migratory journey to the Sargasso Sea for spawning. Additionally, this LWR can facilitate the identification of potential variations among different stock units of the same species or highlight differences between sexes [9].

MATERIALS AND METHODS

Data Collection
Site and Sampling: The study was conducted in the Karavasta lagoon, Albania. A total of 140 individuals were collected using a fyke net by the end of December 2023.
*Biological Parameters***:** Each specimen was assessed for Total Length, which varied from a minimum of 34.9 cm to a maximum of 61.7 cm, yielding a mean of 45.93 cm, and for Weight, which ranged from 76 g to 452 g, with an average of 171 g. The sample comprised 140 silver eels with a sex distribution of 56 males and 84 females.
*Data Analysis***:** Length-frequency histograms were constructed by categorizing individuals into 1 cm length intervals, revealing a bimodal distribution within the dataset. The sex ratio was determined, highlighting a significant sexual dimorphism, with a threshold indicating that all individuals exceeding 44 cm in length were females. The relationship between length and weight was analyzed, indicating negative allometric growth, which was particularly evident when the Length-Weight relationship was examined separately for each sex.

RESULTS AND DISCUSSION

The results of this research uncovered several noteworthy findings:

- A bimodal distribution of lengths within the sample.
- A distinct sexual dimorphism, characterized by a clear breakpoint at 44 cm total length, above which all individuals were identified as female.
- Evidence of negative allometric growth, particularly evident when analyzing the Length-Weight relationship by sex.

- The health evaluation of the migrating silver eels demonstrated optimal health, as none of the 140 individuals were found to be infected with the *Anguillicola crassus* nematode.

The observed bimodal length distribution and the marked sexual dimorphism observed, with females predominating the larger size category, are typical traits of *Anguilla anguilla*. This sexual dimorphism may be associated with the eel's intricate life cycle, wherein females undertake migration to the Sargasso Sea for reproductive purposes, necessitating a larger body size.

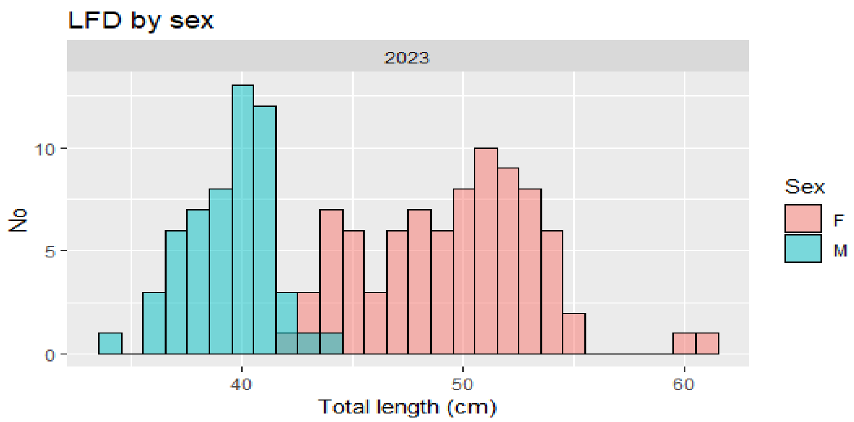


Table 1: Length frequency distribution (LFD) by sex

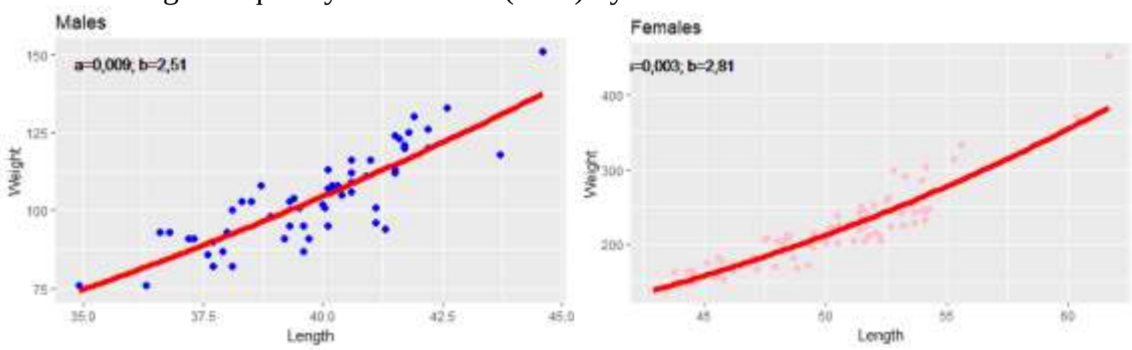


Table 2&3: Length-Weight relationship divided by sex

The lack of *A. crassus* nematode infection in the migrating silver eels indicates a positive health status for this population. This observation is encouraging for the conservation efforts of European eels in the Karavasta lagoon.

CONCLUSION

This research marks an important advancement in the comprehension of the local stock of European eels within the Karavasta lagoon in Albania. The results indicating a bimodal length distribution and distinct sexual dimorphism offer critical insights into the population dynamics of this species, which is classified as critically endangered. Moreover, the favorable health status noted among migrating silver eels serves as an encouraging indicator for their conservation efforts. It is essential that additional studies be undertaken to achieve a more thorough understanding of the local stock in the Karavasta lagoon, thereby aiding in the responsible management of European eel populations.

REFERENCES

W. Dekker. “Status of the European eel stock and fisheries." *Eel biology*. Tokyo: Springer Japan,. 237-254, 2003.

Tesch FW. “The Eel”. Wiley-Blackwell Publishing, Oxford, 2003.

C. Belpaire, P. Hodson, F. Pierron, M. Freese. Impact of chemical pollution on Atlantic eels: Facts, research needs, and implications for management. *Current Opinion in Environmental Science & Health*, 11:26-36, 2019.

D. Jacoby, and M. Gollock. "Anguilla anguilla. IUCN 2014. IUCN Red List of Threatened Species. Version 2014.1.", 2014.

W. Dekker. "A Procrustean assessment of the European eel stock." *ICES Journal of Marine Science* 57.4: 938-947, 2000.

J. Simon. "Age, growth, and condition of European eel (Anguilla anguilla) from six lakes in the River Havel system (Germany)." *ICES Journal of Marine Science* 64.7 (2007): 1414-1422.

European Union (EU). Council Regulation (EC) No. 1100/2007 Establishing measures for the recovery of the stock of European eel, 2007.

V. Aschonitis, G. Castaldelli, M. Lanzoni, R. Rossi, C. Kennedy, and E.A. Fano, E. A. “Long-term records (1781–2013) of European eel (Anguilla anguilla L.) production in the Comacchio Lagoon (Italy): Evaluation of local and global factors as causes of the population collapse”. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*(2), 502-520, 2017.

M. King. “Fisheries biology, assessment and management. Second Studia Marina” Blackwell Scientific Publications, Oxford. 35 (2): 5-11 10 Edition, 2007.

THE EFFECT OF PHOSPHORUS FERTILISATION ON ESSENTIAL OIL YIELD OF LAVENDER PLANT (*LAVANDULA ANGUSTIFOLIA MILL.*) IN CALCAREOUS SOILS

Gülbeyaz DERIN ALTAY [1,], Sevgi ÇALIŞKAN [1]*

[1]*Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Nigde Omer Halisdemir University, Niğde, Türkiye*

**Correspondence: glbyz51kbr@hotmail.com*

Gülbeyaz DERIN ALTAY: https://orcid.org/0000-0002-2694-1162
Sevgi ÇALIŞKAN: https://orcid.org/0000-0001-8491-4894

ABSTRACT

The primary purpose of our study is to determine the effects of different doses of phosphorus fertilization on the development and essential oil yield of lavender and to determine the phosphorus doses required to obtain the best yield in the *Lavandula Angustifolia* mill. variety. The study was established and conducted in Niğde Ömer Halisdemir University, Faculty of Agricultural Sciences and Technologies, medicinal plants application area according to the randomized blocks experimental design with three replications. The experimental area established with lavender *Angustifolia Sevtapolis* species was divided into plots as P0: 0 kg P_2O_5/Da, P6: 6 kg P_2O_5/Da, P12: 12 kg P_2O_5/Da, P18: 18 kg P_2O_5/Da, P24: 24 kg P_2O_5/Da, and phosphorus fertilization in the form of triple superphosphate (42-44%) was applied twice before lavender planting and in March 2023 in accordance with the experimental design. The average plant height of lavender was 59,997cm, and the longest plant height was 61,522cm in the P6 group. The shortest plant height was 56,289cm in the P0 group. In our study, the average fresh and dry flower yields of lavender plants were 131.48kg/ha and 111.22kg/ha, respectively. The highest fresh and dry flower yields were 144,49kg/da and 121,60kg/da in P6 group, while the lowest yields were 122,33kg/da and 102,87kg/da in P0 group. In lavender plants with an average essential oil yield of 3,757kg/da, the highest essential oil yield was found in P6 group (4,550kg/da) and the lowest essential oil yield was found in P0 group (3,182kg/da) and the difference between the groups was significant ($p<0,001$).

Key words: Lavender, Angustifolia Sevtapolis, phosphorus fertilization, essential oil, yield.

INTRODUCTION

Lavandula (*Lavandula angustifolia* mill.), also known as English lavender or true lavender, one of more than 50 varieties of the lavandula genus belonging to the *Lamiaceae* family, consisting of aromatic plants such as mint, rosemary and basil, originates from the Mediterranean region (Erland & Mahmoud, 2016). Lavender, a valuable essential oil plant, is a perennial and semi-shrub-like plant (Guenther, 1952). This shrub is frequently preferred in lavender cultivation for use in medical applications due to the sedative, antidepressant and anti-inflammatory effects of the essential oils it contains (Denner, 2009; Jianu et al., 2013). In various studies, the essential oil yield of lavender was determined between 0.2-4.5% and in studies on the effects of phosphorus fertilization on *Lavandula angustifolia* plant, results were found that phosphorus fertilization increased the essential oil yield of lavender (Peçanha et al., 2021). The primary purpose of our study is to determine the effects of different doses of phosphorus fertilization on the development and essential oil yield of lavender and to determine the phosphorus doses required to obtain the best yield in the *Lavandula Angustifolia* mill. variety. *Lavandula angustifolia*, which can grow in any soil that is not overly acidic and well-drained, reaches the highest essential oil yield in light sandy soil and its scent is more pronounced in calcareous soil (Lim, 2012). Lime application to the soil improves the soil by providing neutralization and positively affects the development of lavender (Valcheva, 2020). In line with this information, we preferred a field with alkaline (pH:7,8) and high calcareous soil (42,1%) for our study. In the studies on phosphorus fertilization of lavender in the existing literature, the characteristics of the soil used in the experiments were not frequently mentioned. This may be related to the significant essential oil yield results obtained from measurements on the same variety of lavender (*Lavandula angustifolia* mill.) plants (Peçanha et al., 2021). Therefore, the secondary aim of our study was to characterize the soil used in the experiment and to discuss our results with respect to the characteristics of the experimental soil.

MATERIALS AND METHODS

1.1. Plant materials

Lavandula Angustifolia Sevtapolis variety was used as plant material. The seedlings used in the study were cut from clonal rootstocks and produced in the Fruit Research Institute Directorate (Isparta/Turkey). These seedlings were supplied as 1+1-year-old bare root seedlings.

1.2. Experimental design

The study was established and conducted with three replications according to the Randomized Complete Blocks Design at Niğde Ömer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Medicinal Plants Application Area (37.94” North and 34.62” West, Turkey). The region has typical continental climate characteristics. The average climate data of Nigde province, where the study was conducted, is given in table 1.

Table 1. The average climate data of Nigde province

Months	Avarage Tempature (°C)			Total montly rainfall (mm)			Average relative humidity (%)		
	2022	2023	Avarage of 1991-2021	2022	2023	Avarage of 1991-2021	2022	2023	Avarage of 1991-2021
January	-1.8	1.9	0.1	30.2	11.4	35.1	71.1	65.4	73.2
February	1.9	-1.2	1.4	47.2	23.2	31.3	71.0	68.8	68.8
March	0.5	7.7	6.0	37.6	43.0	38.6	67.6	60.7	61.9
April	13.8	10.6	11.0	3.6	62.4	40.1	39.1	57.4	56.4
May	14.3	14.9	15.7	57.6	23.6	42.1	56.4	54.9	55.3
June	19.9	19.1	19.8	46.0	55.4	29.9	52.3	58.2	50.3
July	21.9	23.0	23.2	4.8	13.0	5.3	43.2	39.7	42.9
August	25.4	25.8	23.1	0.0	11.8	7.8	38.2	33.8	43.8
September	19.7	19.7	18.8	17.4	9.8	12.7	43.0	41.5	47.8
October	13.2	14.4	13.3	3.8	13.6	29.3	57.3	53.8	57.5
November	8.1	9.8	6.6	29.4	14.6	32.6	64.7	61.1	66.3
Decemer	4.7	5.7	2.0	9.4	40.0	43.1	74.9	69.3	73.1
Avarage	11.8	12.6	11.7				56.6	55.4	58.1
Total				287.0	321.8	348.0			

This meteorological data were obtained from Nigde provincial meteorology directorate.

Before the establishment of the experiment, the field soil was analyzed at Soil and Water Analysis Laboratory of Nigde Special Provincial Administrationand and it was found that the saturation was 41.8%, pH 7.8, salt content 0.01%, lime ($CaCO_3$) content 42.1%, organic matter content 0.2%, available phosphorus (P_2O_5) content 2.4 Kg/Decar. The experimental area was divided into plots as P0: 0 kg P_2O_5/ha, P6: 6 kg P_2O_5/ha, P12: 12 kg P_2O_5/ha, P18: 18 kg P_2O_5/ha, P24: 24 kg P_2O_5/ha, and phosphorus fertilization in the form of triple superphosphate (42-44%) was applied twice before planting lavandula and in March 2023 in accordance with the experimental design. The seedlings were planted in the experimental field in March 2022 with a distance of 120 cm between rows and 50 cm above rows (McGimpsey & Porter, 1999). Irrigation was done at two-day intervals and 4 hours a day using drip irrigation system. Pure nitrogen fertilization was applied to the field twice a year at a dose of 10 kg/da.

1.3. Measurements

The first harvest of lavender was made in August 2022, and the first harvest data were not taken into consideration in the evaluation of the yield potential of lavender, which is a perennial plant, since the second harvest data are more appropriate (Giannoulis et al., 2020). Before the second harvest, morphological measurements (plant height, habitus diameter, number of flower spikes) were made. Immediately after the morphological measurements, lavender plants were cut 10 cm above the soil in August 2023 and harvested for the second year (Hassiotis et al., 2014). Wet weight and flower yield (kg/ha) were measured without waiting after harvest. Plants were left to dry in a dark place at temperature. After drying, dry weight and dry flower yield (kg/ha) were measured.

For each plot, 25 g of dried flower samples were taken for oil analysis by Batı Akdeniz Tarımsal Araştırma Enstitüsü Müdürlüğü (BATEM) and essential oils of the samples were obtained by water vapor distillation method in Clevenger device and essential oil ratios were calculated as percentage (Skoula et al., 2000). Basic scent components of the essential oils were analyzed by GC/GC-MS (Gas chromatography (Agilent 7890A)-mass detector (Agilent 5975C)) using a capillary column (HP Innowax Capillary; 60.0 m x 0.25 mm x 0.25 µm). The amounts of phenolic substances were determined as gallic acid equivalent (mgGAE/100g) using the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965).

1.4. Statistical analyses

In the analysis of the data, the results of morphological measurements and chemical analyses obtained from the plots divided in coincidence blocks in accordance with the field experiment design were given as mean, standard deviation, minimum and maximum. The differences between the groups treated with different doses of phosphorus were tested by one-way analysis of variance (ANOVA). The interactions of the parameters showing statistically significant differences as a result of one-way analysis of variance with phosphorus dose were examined by quadratic regression analyses and effective phosphorus fertilization doses were calculated. Correlation analyses were performed to determine whether there is a relationship between the data obtained in the study. All data were analyzed with 95% confidence interval and IBM SPSS Statistics for Windows (Ver22.0, IBM Corp., Armonk, NY: USA, 2013) was used for data analysis.

2. RESULTS

In the 1st and 2nd year, soil analysis was repeated after all fertilization treatments were applied. Saturation was 36.45%, pH 7.67, salt content 0.01%, lime ($CaCO3$) content 37.91%, organic matter content 1.97%, available phosphorus ($P2O5$) content 31.26 kg/decar.

Descriptive data of the study parameters and the results of the analysis of variance of the study parameters between the groups administered different doses of phosphorus are presented in Table 2.

Table 2. Mean, standart deviation, minimum and maximum datas of the lavander plants and the results of one way variance analysis between the different phosphorus dose groups.

	Descriptives				Differences between the groups	
	Min.	**Max.**	**Mean**	**S.D.**	**F**	**p-value**
Plant Height (cm)	52.90	68.40	60.00	4.11	2.88	0.035
Habitus Diameter (cm)	47.70	75.30	62.72	7.92	7.69	<0.001
Number of FS (pcs/plant)	78.30	126.80	100.45	13.89	3.78	0.011
Fresh Flower Yield (kg/da)	117.20	169.90	131.48	12.16	6.00	0.001
Dried Flower Yield (kg/da)	98.40	144.20	111.22	10.12	5.83	0.001
Essential Oil Yield (kg/da)	2.37	5.22	3.76	0.59	14.70	<0.001
Amount of PS (GAE/100g)	0.43	1.39	0.93	0.15	2.20	0.087
Antioxidant Activity (µL)	0.94	2.98	1.94	0.40	3.05	0.028

Min: minimum, Max: maximum, SD: Standart deviation, Sig.: Significance, FS: Flower spikes, PS: Phenolic substances

2.1. Correlation analyses

Correlation analysis was performed between the study parameters and the results of correlation analysis are given in table 3. There was a strong positive correlation between plant height, habitus diameter and number of flower spikes and wet and dry yields. There is a strong positive correlation between essential oil yield (kg/ha) and habitus diameter and a positive correlation between essential oil yield (kg/ha) and number of flower spikes.

Table 3. Correlation between the study parameters.

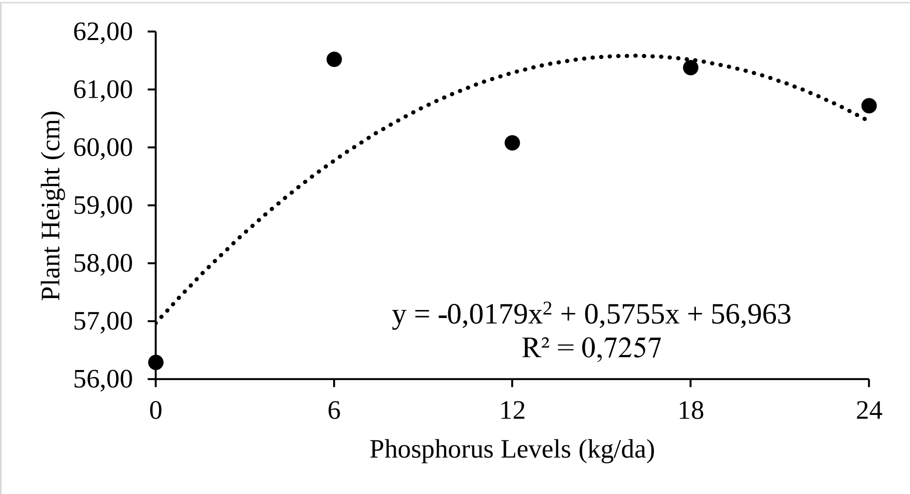
	P. Dose	Plant Height	Habitus Diameter	Number of FS	Fresh Flower Yield	Dried Flower Yield	EOY (kg/da)	Amount of PS
Plant Height	0.303*	1						
Habitus Diameter	0.028	0.793**	1					
Number of FS	0.336*	0.826**	0.807**	1				
Fresh Flower Yield	0.067	0.536**	0.536**	0.585**	1			
Dried Flower Yield	0.098	0.533**	0.537**	0.615**	0.986**	1		
EOY (kg/da)	-0.037	0.266	0.414**	0.321*	0.729**	0.743**	1	
Amount of PS	-0.081	0.049	0.107	0.186	-0.131	-0.132	-0.134	1
AO Activity	0.280	-0.204	-0.347*	-0.258	-0.060	-0.055	-0.278	-0.367*

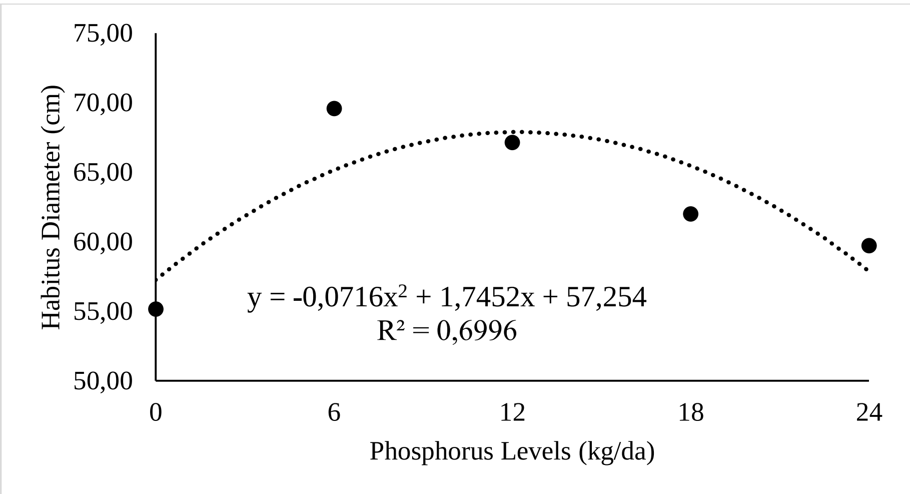
P. Dose: Phosphorus Dose, FS: Flower spikes, PS: Phenolic Substances, EOY: Essential oil yield, AO: Antioxidant

*: Correlation is significant at the 0.05 level (2-tailed), **: Correlation is significant at the 0.01 level (2-tailed)

2.2. Regression analyses

The average plant height of lavender was 59,997±4,114 cm, the longest plant height was 61,522±1,143 cm in P6 group. The shortest plant height was 56,289±2,761 cm in P0 group. The effect of phosphorus applied at different doses on plant height was significant (p=0.035) and quadratic regression analysis was performed between them (y = -0.0179x2 + 0.5755x + 56.963, R2=0.7257, Figure 1). According to this regression, the effective phosphorus dose required to obtain the longest plant height was calculated as 16,075 kg/ha. The average habitus diameter was 62,724±7,922cm, the widest was 69,589±4,788cm in P6 group and the narrowest was 55,156±8,344cm in P0 group. The difference in habitus diameter between the groups was significant at p<0.001 level and the effective phosphorus dose for the widest habitus diameter was determined as 12.185kg/ha (y = -0.0716x2 + 1.7452x + 57.254, R² = 0.6996, Figure 1). The average number of flower spike of lavender plants was 100,45±20,25. The highest number of flower spike was 105,51±3,47 in P12 group and the lowest number of flower spike was 86,18±6,57 in P0 group and the difference between the groups was significant (p=0,011). The effective phosphorus dose required to reach the maximum number of flower spike was calculated as 15,315kg/ha (y = -0,0821x2 + 2,5148x + 88,009, R² = 0,8844, Figure 1).





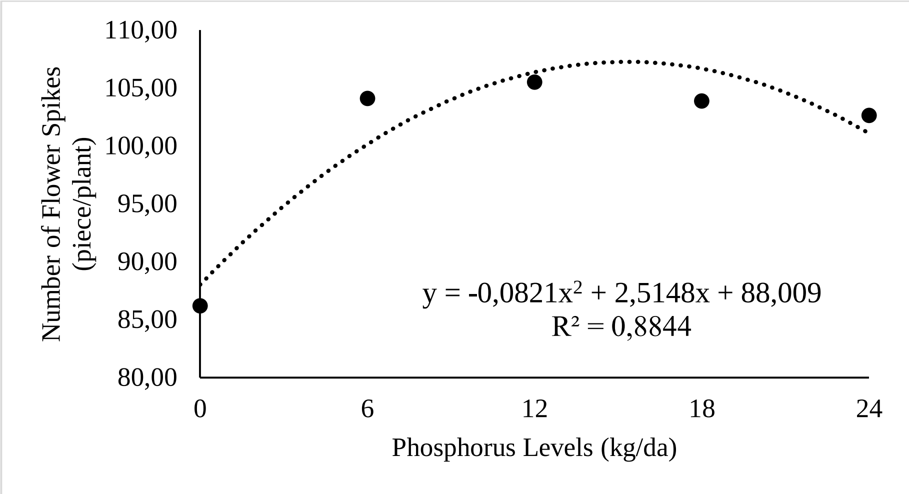


Figure 1. Regression analysis between phosphorus doses and plant height, habitus diameter, number of flower spikes.

In our study, the average fresh and dry flower yields of lavender plants were 131,48±12,16kg/da and 111,22±10,12kg/da, respectively. The highest fresh and dry flower yields were 144,49±12,19kg/da and 121,60±9,87kg/da in P6 group, while the lowest yields were 122,33±3,79kg/da and 102,87±3,35kg/da in P0 group. The differences between fresh and dry flower yields were statistically significant at p=0.001 level. The effective phosphorus dose to obtain the best fresh flower yield was calculated as 13,197kg/da ($y = -0{,}0394x2 + 1{,}0399x + 127{,}51$, $R^2 = 0{,}1158$) and the effective phosphorus dose to obtain the best dry flower yield was calculated as 13,442kg/da ($y = -0{,}0397x2 + 1{,}0673x + 106{,}98$, $R^2 = 0{,}1809$) (Figure 2). In lavender plants with an average essential oil yield of 3,757±0,593kg/ha, the highest essential oil yield was found in P6 group (4,550±0,354kg/ha) and the lowest essential oil yield was found in P0 group (3,182±0,602kg/ha) and the difference between the groups was significant ($p<0{,}001$). The effective phosphorus fertilization dose to be applied to lavandula to obtain the highest essential oil yield was calculated as 11,680kg/ha ($y = -0{,}0047x2 + 0{,}1098x + 3{,}4478$, $R^2 = 0{,}3909$, Figure 2). The mean phenolic content of lavender was 0,925±0,149GAE/100g. The highest phenolic content was measured in P12 group lavender with 0,999±0,189GAE/100g and the lowest phenolic content was measured in P6 group lavender with 0,833±0,173GAE/100g. There was no statistically significant difference in phenolic content between the groups (p=0,087). The average antioxidant activity (EC50) of lavandulas was 1,937±0,396µL, the highest antioxidant activity was found in P24 group (2,239±0,414µL) and the lowest antioxidant activity was found in P6 group (720±0,212µL). There was a significant difference between the groups (p=0,028). Although 9.52kg/da phosphorus dose application was predicted to cause the lowest antioxidant activity ($y = 0.0025x2 - 0.0476x + 1.9625$, $R^2 = 0.948$), no significant correlation was found between the phosphorus doses applied, wet yield, dry yield and essential oil yield.

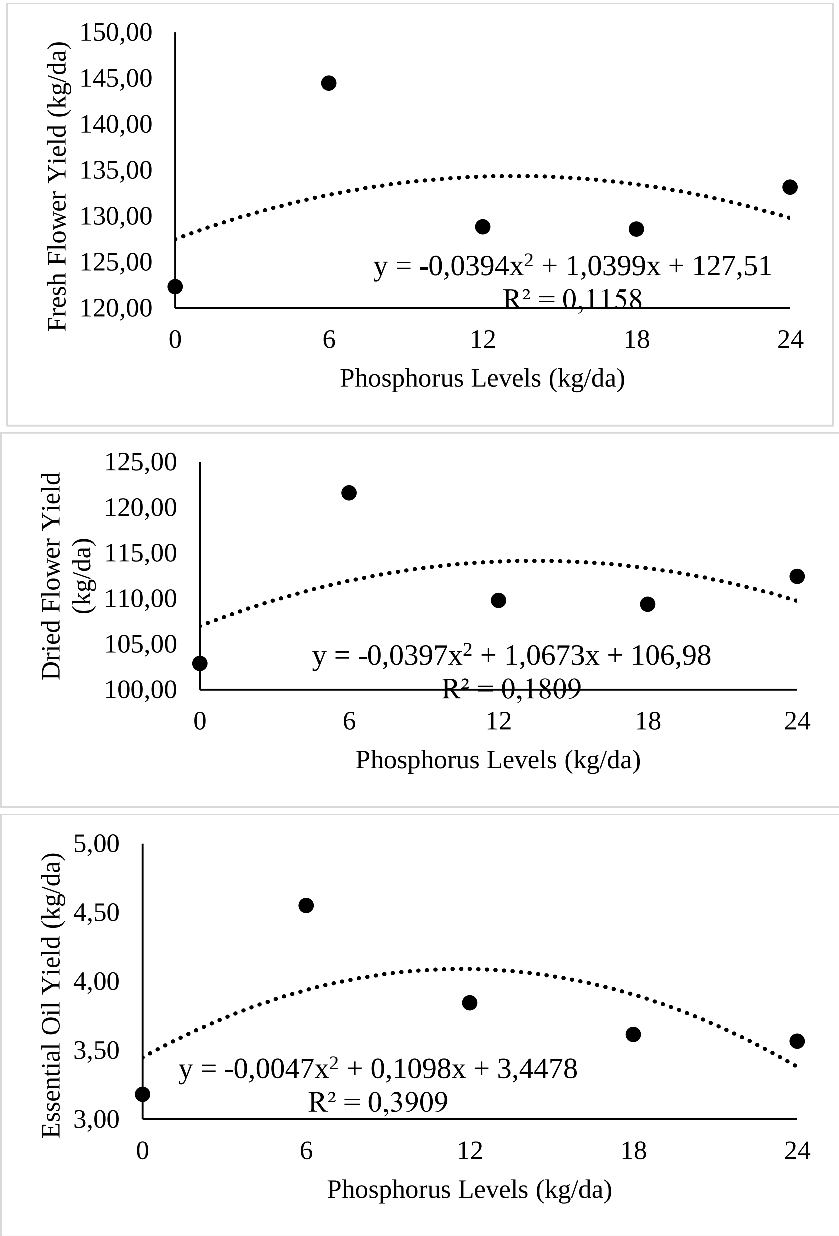


Figure 2. Regression analysis between phosphorus doses and fresh flower yield, dry flower yield, essential oil yield.

DISCUSSION

Although it has been classically shown that the productivity of lavender increases at pH 6.4 (neutral) and pH 8.2 (slightly alkaline) acidity values (Crișan et al., 2023), recent studies have shown that soil pH should be between 6.5 and 7.5 for good development of lavender (Crummitt & Drost, 2012). The lime content of the soil in the experimental field where our study was conducted was as high as 42.1% and accordingly, the pH value was measured as 7.8 before the application. Even though this lime content was 37.9% and pH was 7.67 after fertilisation, pH did not fall below 7.5 as a result of the high lime content of the experimental soil. Nevertheless, the data obtained in our study were generally similar to the literature and this situation suggests that the high pH and lime content of the soil does not have a negative effect on lavender growth. Although lavender plant height is related to the genetic potential of the variety, it is also known to be affected by different growth environment or environmental factors (Arabaci & Bayram, (2005). Although lavender can grow up to 1 metre in length under optimum growth conditions, similar to our study, in other studies, researchers reported that plant height varied between 60.4-69.5 cm (Arabaci & Bayram, (2005), 43.2-79.5 cm (Chrysargyris, 2018) and 40.8 cm in another study (Özyilmaz et al., 2022). In our study, the longest plant height was obtained in P6 group and the shortest plant height was obtained in P0 group. Erbaş et al., 2017 investigated the effects of 0, 5, 10 and 15 kg/da phosphorus applications on lavandin and the longest plant height was measured in the group fertilized at 10 kg/da (104.7 cm) and the shortest plant height was measured in the control group (93.0 cm). In the same study, plant height was measured as 94.3 cm in the group with 15 kg/da phosphorus fertilization (Erbaş et al., 2017). In our study, the phosphorus dose required to reach the maximum plant height was found to be 16.075 kg/da. In our study, the habitus diameter of lavender plants was found to be the widest in P6 group and the narrowest in P0 group parallel to plant height. In another study investigating the effects of different types of fertilizations on lavender growth, habitus diameters were measured and it was observed that habitus diameters were parallel to plant height (Pryvedeniuk et al., 2023). In our study, a strong positive correlation was shown between plant height and habitus diameter measurements for Lavandula angustifolia. We also found that habitus diameter showed a strong positive correlation with essential oil content and according to our results, habitus diameter was the most effective morphological measurement to show the essential oil content of Lavandula angustifolia. The number of flower spike, which is determined by counting the spikes formed on the flower stalks of the plant, is one of the important parameters affecting flower yield and may vary according to species and varieties (Karık et al., 2017). Researchers found that the number of flower spikes varied between 55.9 and 471.9 pieces/plant (Aslan & Sarihan, 2021). In our study, the average number of flower spike was 100.45±20.25, the highest number of flower spike was determined in P12 group and the lowest number of flower spike was determined in P0 group and it showed positive correlation with other morphological parameters. In the other two studies in which different treatments were applied, fresh stem flower yield in lavender varied between 462.6-613.8 kg/da (Kara & Baydar, 2013) and 541.7-569.9 kg/da (Karık et al., 2017), dry stem flower yield varied between 219.7-312.0 kg/da (Kara & Baydar, 2013), 85.6-450.7 kg/da (Karık et al., 2017) and 185.0-366.0 kg/da (Pistelli et al., 2017). Erbaş et al., who investigated the effects of phosphorus application at different doses, found that both dry and fresh flower yields of plants treated with 10kg/da phosphorus were significantly higher than the other groups, while the control group was significantly lower than the other groups (Arabaci & Bayram, (2005). These results were similar to our results. In our study, the average fresh and dry flower yields of lavender plants were 131.48±12.16kg/da and 111.22±10.12kg/da, respectively, and the highest fresh and dry flower yields were measured in P6 group and the lowest fresh and dry flower yields were measured in P0 group. In our study, it was calculated that phosphorus application at doses of 13,197kg/da for the highest fresh flower yield and 13,442kg/da for the highest dry flower yield. In previous studies, it was reported that the essential oil yield in lavender varied between 3.17-4.13 kg/da (Ceylan et al., 1988), and in Lavandula angustifolia, the essential oil yield varied between 1.49-2.53 kg/da (Atalay, 2008) and 0.83-13.47 kg/da (Arabaci & Bayram, (2005) in two different studies. In our study, in lavender plants with an average essential oil yield of 3,757±0,593kg/da, the highest essential oil yield was found in P6 group (4,550±0,354kg/da) and the lowest essential oil yield was found in P0 group (3,182±0,602kg/da) and the difference between the groups was significant ($p<0,001$). In the study of Erbaş et al., 2.09% and 2.10% essential oil content was determined in 15kg/da and 10kg/da phosphorus dose groups, respectively, and it was significantly different from the control group (Erbaş et al., 2017). In our study, the phosphorus dose required to obtain the best essential oil yield was calculated as 11,680kg/da. In another study conducted as a pot experiment under tropical conditions, regression curves and effective doses were calculated and the ideal phosphorus dose for the best essential oil yield was found to be 77 mg/dm3 (Peçanha et al., 2021). However, this study is different from our study both because it was a pot study and because the climatic conditions were kept under controlled conditions.

In conclusion, lavender has adapted to the slightly alkaline soil with high lime content of our trial area. Phosphorus fertilization is known to be beneficial for Lavandula angustifolia. In this study, in which we aimed to find out this beneficial effect in more detail, we calculated by means of regression graphs that phosphorus should be applied at a dose of 13,197kg/da for maximum fresh flower yield, 13,442kg/da for maximum dry flower yield and 11,680kg/da for maximum essential oil yield. Our study stands out as one of the pioneering field studies in which Lavandula angustifolia effective phosphorus dose calculation was made by regression analyses.

ACKNOWLEDGMENT

We acknowledge to Niğde Provincial Meteorology Directorate for providing meteorological data between 1991-2023.

REFERENCES

Arabacı, O., & Bayram, E. (2005). Aydın ekolojik koşullarında lavanta (Lavandula Angustifolia mill.)’Nın bazı agronomik ve kalite özellikleri üzerine bitki sıklığı ve azotlu gübrenin etkisi. Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi, 2(2), 13-19.

Aslan, S., & Sarıhan, E. O. (2021). Humik asit ve azotlu gübre uygulamalarının lavanta (Lavandula angustifolia Mill.) bitkisinin bazı verim ve kalite özelliklerine etkisi. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 26(1), 29-40.

Atalay, A. T. (2008). Konya ekolojik şartlarında yetiştirilen lavanta (Lavandula angustifolia mill.)'da farklı dozlarda uygulanan organik ve inorganik azotlu gübrelerin verim ve kalite özellikleri üzerine etkileri.

Ceylan, A., Vömel, A., Kaya, N., Çelik, N., & Niğdeli, E. (1988). Bitki sıklığının lavanta'da verim ve kaliteye etkisi üzerinde araştırma. EÜ Zir. Fak. Dergisi, 25(2), 135-145.

Chrysargyris, A., Michailidi, E., & Tzortzakis, N. (2018). Physiological and biochemical responses of Lavandula angustifolia to salinity under mineral foliar application. Frontiers in plant Science, 9, 489.

Crişan, I., Ona, A., Vârban, D., Muntean, L., Vârban, R., Stoie, A., ... & Morea, A. (2023). Current trends for lavender (lavandula angustifolia Mill.) crops and products with emphasis on essential oil quality. Plants, 12(2), 357.

Crummitt, K., & Drost, D. (2012). English Lavender in the Garden. USA, Utah State, published of May, 8.

Denner, S. S. (2009). Lavandula angustifolia miller: english lavender. Holistic Nursing Practice, 23(1), 57-64.

Erbaş, S., Kucukyumuk, Z., Baydar, H., Erdal, I., & Sanli, A. (2017). Effects of different phosphorus doses on nutrient concentrations as well as yield and quality characteristics of lavandin (Lavandula× intermedia Emeric ex Loisel. var. Super). Turkish Journal of Field Crops, 22(1), 32-38.

Erland, L. A., & Mahmoud, S. S. (2016). Lavender (Lavandula angustifolia) oils. Essential oils in food preservation, flavor and safety, 501-508.

Giannoulis, K. D., Evangelopoulos, V., Gougoulias, N., & Wogiatzi, E. (2020). Could bio-stimulators affect flower, essential oil yield, and its composition in organic lavender (Lavandula angustifolia) cultivation?. Industrial Crops and Products, 154, 112611.

Guenther, E. (1952). The essential oils. R.E. Krieger Pub. Co. 5: 3-38.

Hassiotis, C. N., Ntana, F., Lazari, D. M., Poulios, S., & Vlachonasios, K. E. (2014). Environmental and developmental factors affect essential oil production and quality of Lavandula angustifolia during flowering period. Industrial crops and products, 62, 359-366.

Jianu, C., Pop, G., Gruia, A. T., & Horhat, F. G. (2013). Chemical composition and antimicrobial activity of essential oils of lavender (Lavandula angustifolia) and lavandin (Lavandula x intermedia) grown in Western Romania. Int. J. Agric. Biol, 15(4), 772-776.

Kara, Nımet., & Baydar, Hasan. (2013). Influence of distillation time and fractions on essential oil content and composition of lavandin (Lavandula× intermedia Emeric ex Loisel.). Crops, 14, 1128-1134.

Karık, Ü., Çiçek, F., & Çınar, O. (2017). Determination of morphological, yield and quality characteristics of Lavandula species and cultivars in Menemen ecological conditions. Anadolu, 27(1), 17-28.

Lim, T. K. (2012). Edible medicinal and non-medicinal plants (Vol. 1, pp. 656-687). Dordrecht, The Netherlands:: Springer.

McGimpsey, J. A., & Porter, N. G. (1999). Lavender: A Growers' Guide for Commercial Production. Publications Section, New Zealand Institute for Crop & Food Research Limited.

Özyılmaz, B., Kadakoğlu, F., Ahmet, B. O. Z., & Karataş, R. (2022). Tokat ekolojik koşullarında farklı Lavandula Angustifolia çeşitlerinin bazı bitkisel özellikleri ve uçucu yağ oranları. 29 September–02 October Rize Türkiye, 58.

Peçanha, D. A., Freitas, M. S. M., Vieira, M. E., Cunha, J. M., & de Jesus, A. C. (2021). Phosphorus fertilization affects growth, essential oil yield and quality of true lavender in Brazil. Industrial Crops and Products, 170, 113803.

Pistelli, L., Najar, B., Giovanelli, S., Lorenzini, L., Tavarini, S., & Angelini, L. G. (2017). Agronomic and phytochemical evaluation of lavandin and lavender cultivars cultivated in the Tyrrhenian area of Tuscany (Italy). Industrial Crops and Products, 109, 37-44.

Pryvedeniuk, N., Hlushchenko, L., Kutsyk, T., Shatkovskyi, A., Shatkovska, K., & Shevchenko, T. (2023). Influence of mıneral fertılızers and plantıng densıty on the growth, development and yıeld of narrow-leaved lavender (Lavandula angustifolia Mill). Poljoprivreda i Sumarstvo, 69(2), 165-180.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158.

Skoula, M., Abbes, J. E., & Johnson, C. B. (2000). Genetic variation of volatiles and rosmarinic acid in populations of Salvia fruticosa mill growing in Crete. Biochemical systematics and ecology, 28(6), 551-561.

Valcheva, V. (2020). Influence of the liming on the soil and morphological indicators of lavender grown in organic agriculture. KNOWLEDGE-International Journal, 41(4), 759-770.

THE AGRICULTURAL USE OF BIOMASS: TRANSFORMING WASTE INTO RESOURC

Serdar ÜÇOK*[1]

[1]*Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Biosystems Engineering, Kahramanmaras, TURKEY*

ABSTRACT

The agricultural use of biomass represents a vital strategy for enhancing sustainability, improving waste management, and reducing greenhouse gas emissions in farming practices. Biomass, derived from organic materials such as crop residues, animal manure, and dedicated energy crops, can be converted into renewable energy, soil amendments, and other valuable products. This approach not only mitigates environmental impacts but also supports the transition to a circular economy by repurposing agricultural waste. Technologies such as anaerobic digestion, pyrolysis, and fermentation play a crucial role in efficiently converting biomass into bioenergy and bio-based products, contributing to the resilience and productivity of agricultural systems. Furthermore, the adoption of biomass technologies promotes rural economic development, creating jobs and stimulating local economies. However, to fully realize the potential of biomass in agriculture, ongoing research, technological advancements, and supportive policies are essential. This abstract provides an overview of the benefits, challenges, and future prospects of biomass utilization in agriculture, highlighting its role as a cornerstone in the transition towards more sustainable and climate-resilient farming practices.

INTRODUCTION

Agriculture is a cornerstone of human civilization, providing food, fiber, and fuel for centuries (Popp et al, 2014). However, modern agriculture faces significant challenges, including the need for sustainable practices, waste management, and the reduction of greenhouse gas emissions. One promising solution to these challenges is the use of biomass. By transforming agricultural waste into valuable resources, biomass can enhance the sustainability and efficiency of agricultural systems. This article explores the agricultural use of biomass, its benefits, technologies, and future prospects (Sims et al, 2006).

In the face of mounting environmental challenges and the imperative for sustainable agricultural practices, biomass emerges as a potent solution (Popp et al, 2014). Biomass, derived from organic materials such as plants, agricultural residues, and animal waste, has been used by humans for millennia. However, modern advancements in technology have vastly expanded its potential applications, transforming it into a cornerstone of sustainable agriculture (Tshikovhi and Motaung 2023).

Agriculture is both a contributor to and a victim of climate change, generating substantial greenhouse gas emissions while also being highly vulnerable to the impacts of a warming planet. One of the key strategies to mitigate these effects and enhance the sustainability of agricultural practices is the effective use of biomass **(Smith et al, 2007).** By converting agricultural waste into valuable resources, biomass can address issues of waste management, renewable energy production, soil health, and carbon sequestration (Lal, 2004), (McKendry, 2002).

The use of biomass in agriculture not only helps in reducing the environmental footprint of farming activities but also supports the transition to a circular economy (Ghisellini et all, 2016). This approach ensures that waste materials are not merely discarded but are repurposed to create energy, improve soil fertility, and sequester carbon. As global populations grow and demand for food increases, the agricultural sector must innovate to meet these needs sustainably (Van et al, 2011). Biomass offers a versatile and effective means to do so, integrating seamlessly with existing agricultural practices and enhancing their overall sustainability (Tshikovhi and Motaung 2023).

This article delves into the myriad ways biomass can be utilized within agriculture, exploring its sources, conversion technologies, environmental benefits, economic impacts, and future prospects (Sims et al, 2006). By understanding the full scope of biomass's potential, we can better appreciate its role in fostering a more sustainable and resilient agricultural system.

What is Biomass in Agriculture?

In the context of agriculture, biomass refers to organic materials derived from plants and animals that can be used for energy production, soil enhancement, and other applications. Common sources of agricultural biomass include crop residues (such as straw, husks, and stalks), animal manure, and dedicated energy crops (like switchgrass and miscanthus). Additionally, waste from food processing and other agricultural by-products can be considered biomass (McKendry, 2002), (Lewandowski et al, 2003), (Cherubini and Ulgiati, 2010), (Monti and Alexopoulou, 2013).

Benefits of Biomass in Agriculture

1. Waste Management

Agricultural activities generate substantial amounts of organic waste, which, if not managed properly, can contribute to environmental pollution and greenhouse gas emissions. Utilizing this waste as biomass for energy production or soil amendments can significantly reduce the environmental footprint of agriculture. This approach transforms waste into a resource, promoting a circular economy in agricultural practices (Ghisellini et al, 2016).

2. Renewable Energy Production

Biomass can be converted into various forms of renewable energy, such as biogas, bioethanol, and biodiesel. These biofuels can be used to power farm machinery, generate electricity, and provide heat, reducing reliance on fossil fuels and lowering greenhouse gas emissions. For instance, anaerobic digestion of animal manure produces biogas, which can be used for electricity generation or heating, while the digestate can be applied as a fertilizer (McKendry, 2002), (Scarlat et al, 2018), (Weiland, 2010).

3. Soil Health and Fertility

Biomass can enhance soil health and fertility through the application of biochar and compost. Biochar, prodced through the pyrolysis of organic materials, improves soil structure, increases water retention, and enhances nutrient availability. Composting agricultural residues enriches the soil with organic matter, promoting microbial activity and plant growth. These practices contribute to sustainable soil management and increased agricultural productivity (Basu, 2013).

4. Carbon Sequestration

The use of biomass in agriculture can contribute to carbon sequestration, the process of capturing and storing atmospheric carbon dioxide (Tilman et al, 2006). Biochar application, in particular, sequesters carbon in the soil for long periods, reducing the overall carbon footprint of agricultural activities (Lal, 2004). This practice helps mitigate climate change by removing CO_2 from the atmosphere and storing it in stable forms.

Technologies for Biomass Utilization in Agriculture

1. Anaerobic Digestion

Anaerobic digestion is a biological process that breaks down organic matter in the absence of oxygen, producing biogas and digestate (Scarlat et al, 2018). This technology is widely used for the treatment of animal manure and other agricultural residues (Tilvikiene and Malakauskiene, 2019). The biogas produced can be used for energy production, while the nutrient-rich digestate serves as an excellent fertilizer, returning valuable nutrients to the soil (McKendry, 2002), (Weiland, 2010).

2. Pyrolysis

Pyrolysis involves the thermal decomposition of organic materials at high temperatures in the absence of oxygen, resulting in the production of biochar, bio-oil, and syngas. Biochar is particularly valuable for its soil-enhancing properties, while bio-oil and syngas can be used as energy sources. Pyrolysis offers a way to convert agricultural residues into high-value products, contributing to both energy production and soil health (Basu, 2013), (Zhang et al, 2019).

3. Composting

Composting is the aerobic decomposition of organic matter by microorganisms, resulting in the formation of humus-like material. This process can be used to convert crop residues, animal manure, and other organic waste into nutrient-rich compost. Compost improves soil structure, enhances nutrient availability, and promotes healthy plant growth. It is a simple yet effective way to recycle agricultural biomass and improve soil fertility (Bernal et al, .2009), (Insam and de Bertoldi, 2007).

4. Fermentation

Fermentation is a biochemical process that converts sugars in organic materials into alcohols, such as ethanol, through the action of microorganisms. This technology is commonly used to produce bioethanol from crops like corn and sugarcane. Bioethanol can be used as a renewable fuel for vehicles, reducing reliance on fossil fuels and lowering greenhouse gas emissions. Advanced fermentation technologies are also being developed to convert lignocellulosic biomass, such as crop residues, into bioethanol (Balat et al, 2008), (Limayem and Ricke, 2012), (Hahn et al, 2006), (Sanchez and Cardona, 2008), (Zhang, 2013).

Future Prospects of Biomass in Agriculture

The future of biomass in agriculture is promising, with ongoing research and technological advancements expanding its potential applications. Some key areas of development include (Sims et al, 2006):

1. Integration with Other Renewable Energy Sources

Combining biomass with other renewable energy sources, such as solar and wind, can create more resilient and efficient energy systems for farms. For example, integrating biogas production with solar panels can provide a stable and continuous energy supply, even when solar energy is not available (Sokhansanj and Fenton, 2006).

2. Precision Agriculture

The use of precision agriculture technologies can optimize the application of biomass-derived products, such as fertilizers and biochar. Precision agriculture involves the use of sensors, data analytics, and GPS technology to tailor agricultural practices to specific field conditions. This approach can enhance the efficiency and effectiveness of biomass utilization, promoting sustainable and productive farming (Mulla, 2013), (Jha et al, 2019), (Zhang et al, 2002), (de Oliveira et al, 2018), (Bhatt 2016).

3. Advances in Biomass Conversion Technologies

Ongoing research is focused on improving the efficiency and sustainability of biomass conversion technologies. Innovations in anaerobic digestion, pyrolysis, and fermentation can increase the yield and quality of biomass-derived products, making them more economically viable and environmentally friendly. Developing new methods to convert a wider range of biomass feedstocks into valuable products can further expand the potential of biomass in agriculture (Smeets et al, 2007).

4. Policy Support and Incentives

Government policies and incentives play a crucial role in promoting the use of biomass in agriculture. Supportive policies can encourage investment in biomass infrastructure, research, and development. Financial incentives, such as subsidies and tax credits, can make biomass technologies more accessible and affordable for farmers. Additionally, regulations that promote sustainable biomass sourcing and utilization can ensure that the environmental benefits of biomass are fully realized (Scarlat et al, 2015), (.Sims et al, 2015), (IRENA, 2016), (Gunningham and Sinclair, 2002), (Panoutsou and Castillo, 2020).

CONCLUSION

The agricultural use of biomass offers a sustainable and versatile solution to some of the most pressing challenges in modern agriculture. By transforming waste into valuable resources, biomass can enhance waste management, produce renewable energy, improve soil health, and contribute to carbon sequestration (Lal, 2004). The development and adoption of advanced biomass conversion technologies, supported by precision agriculture and favorable policies, can unlock the full potential of biomass in agriculture (Smeets et al, 2007). As we continue to seek sustainable practices and innovative solutions, biomass stands out as a key component in the transition towards a more resilient and sustainable agricultural future (Tshikovhi and Motaung 2023).

The agricultural use of biomass represents a crucial intersection of sustainability, innovation, and economic viability. As we face the dual challenges of feeding a growing global population and mitigating the adverse impacts of climate change, biomass offers a multifaceted solution that addresses both issues simultaneously. The ability to convert agricultural waste into renewable energy and valuable soil amendments not only reduces the environmental footprint of farming practices but also enhances the overall productivity and sustainability of agricultural systems.

The benefits of biomass in agriculture are manifold. From improving waste management and reducing greenhouse gas emissions to enhancing soil health and fertility, the integration of biomass into agricultural practices can lead to significant environmental and economic gains. The application of advanced technologies such as anaerobic digestion, pyrolysis, and fermentation further expands the potential of biomass, allowing for the efficient conversion of a wide range of organic materials into useful products (Zhang et al, 2019).

Moreover, the adoption of biomass technologies can drive rural economic development by creating new job opportunities and stimulating local economies. This is particularly important in agricultural regions, where biomass resources are often abundant. By supporting the development of a bio-based economy, we can promote a more sustainable and equitable distribution of economic benefits (Ghisellini et all, 2016).

However, realizing the full potential of biomass in agriculture requires concerted efforts across multiple fronts (Smeets et al, 2007). Technological advancements must continue to improve the efficiency and sustainability of biomass conversion processes. Policies and incentives are needed to support the adoption of biomass technologies and ensure sustainable sourcing and

utilization of biomass resources. Additionally, education and outreach efforts are essential to raise awareness about the benefits of biomass and encourage its widespread use.
Looking to the future, the integration of biomass with other renewable energy sources and precision agriculture technologies holds great promise. Hybrid energy systems that combine biomass with solar, wind, and other renewables can provide a stable and resilient energy supply for farms. Precision agriculture, with its data-driven approach, can optimize the use of biomass-derived products, enhancing their effectiveness and sustainability.
In conclusion, the agricultural use of biomass is a powerful tool for achieving a more sustainable and resilient agricultural sector. By transforming waste into valuable resources, biomass can help address some of the most pressing environmental and economic challenges facing agriculture today. As we continue to innovate and develop new technologies, the potential of biomass will only grow, offering new opportunities for sustainable development and climate change mitigation. Embracing biomass in agriculture is not just a step towards sustainability; it is a stride towards a future where farming practices are in harmony with the environment, ensuring food security and prosperity for generations to come (Popp et al, 2014).

REFERENCES

Balat, M., Balat, H., & Öz, C. (2008). Progress in bioethanol processing. *Progress in Energy and Combustion Science*, 34(5), 551-573.
Basu, P. (2013). **Biomass Gasification, Pyrolysis and Torrefaction: Practical Design and Theory**. Academic Press. This book provides a comprehensive overview of biomass conversion technologies and their applications.
Bernal, M. P., Alburquerque, J. A., & Moral, R. (2009). Composting of animal manures and chemical criteria for compost maturity assessment: A review. *Bioresource Technology*, 100(22), 5444-5453.
Bhatt, R., Kukal, S. S., Busari, M. A., Arora, S., & Yadav, M. (2016). Sustainability issues on rice–wheat cropping system. *International Soil and Water Conservation Research*, 4(1), 64-74.
Cherubini, F., & Ulgiati, S. (2010). Crop residues as raw materials for biorefinery systems—A LCA case study. *Applied Energy*, 87(1), 47-57.
De Oliveira, A. S., Mullen, R. W., & Raun, W. R. (2018). Sensor-based nitrogen management and the use of precision agriculture in improving nitrogen use efficiency in cereal crops. *Agronomy Journal*, 110(6), 2631-2645.
Ghisellini, P., Cialani, C., & Ulgiati, S. (2016). **A review on circular economy: the expected transition to a balanced interplay of environmental and economic systems**. *Journal of Cleaner Production*, 114, 11-32.
Gunningham, N., & Sinclair, D. (2002). Leaders and Laggards: Next-Generation Environmental Regulation. *Greenleaf Publishing*.
Hahn-Hägerdal, B., Galbe, M., Gorwa-Grauslund, M. F., Lidén, G., & Zacchi, G. (2006). Bio-ethanol–the fuel of tomorrow from the residues of today. *Trends in Biotechnology*, 24(12), 549-556.

Insam, H., & de Bertoldi, M. (2007). Microbiology of the composting process. In *Waste Management Series* (Vol. 8, pp. 25-48). Elsevier.

IRENA (International Renewable Energy Agency). (2016). Renewable Energy Benefits: Measuring the Economics.

Jha, K., Doshi, A., Patel, P., & Shah, M. (2019). A comprehensive review on automation in agriculture using artificial intelligence. *Artificial Intelligence in Agriculture*, 2, 1-12.

Lal, R. (2004). **Soil carbon sequestration impacts on global climate change and food security**. *Science*, 304(5677), 1623-1627.

Lewandowski, I., Scurlock, J. M. O., Lindvall, E., & Christou, M. (2003). The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. *Biomass and Bioenergy*, 25(4), 335-361.

Limayem, A., & Ricke, S. C. (2012). Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues, and future prospects. *Progress in Energy and Combustion Science*, 38(4), 449-467.

McKendry, P. (2002). **Energy production from biomass (part 1): overview of biomass**. *Bioresource Technology*, 83(1), 37-46.

Monti, A., & Alexopoulou, E. (Eds.). (2013). Biomass Production and Uses. *CABI Publishing*.

Mulla, D. J. (2013). Twenty-five years of remote sensing in precision agriculture: Key advances and remaining knowledge gaps. *Biosystems Engineering*, 114(4), 358-371.

Panoutsou, C., & Castillo, A. C. (2020). The Role of Policy in the Development of Sustainable Biomass Value Chains. *Frontiers in Energy Research*, 8, 50.

Popp, J., Lakner, Z., Harangi-Rákos, M., & Fári, M. (2014). The effect of bioenergy expansion: Food, energy, and environment. *Renewable and Sustainable Energy Reviews*, 32, 559-578. (Popp et al, 2014).

Sanchez, O. J., & Cardona, C. A. (2008). Trends in biotechnological production of fuel ethanol from different feedstocks. *Bioresource Technology*, 99(13), 5270-5295.

Scarlat, N., Dallemand, J. F., Monforti-Ferrario, F., & Banja, M. (2015). Renewable energy policy framework and bioenergy contribution in the European Union – An overview from National Renewable Energy Action Plans and Progress Reports. *Renewable and Sustainable Energy Reviews*, 51, 969-985.

Scarlat, N., Dallemand, J. F., & Fahl, F. (2018). Biogas: Developments and perspectives in Europe. *Renewable Energy*, 129, 457-472.

Smeets, E. M., Faaij, A. P., Lewandowski, I., & Turkenburg, W. C. (2007). A bottom-up assessment and review of global bio-energy potentials to 2050. *Progress in Energy and Combustion Science*, 33(1), 56-106.

Sims, R. E., Hastings, A., Schlamadinger, B., Taylor, G., & Smith, P. (2006). **Energy crops: current status and future prospects**. *Global Change Biology*, 12(11), 2054-2076.

Sims, R. E. H., Mabee, W., Saddler, J. N., & Taylor, M. (2010). An overview of second-generation biofuel technologies. *Bioresource Technology*, 101(6), 1570-1580.

Sokhansanj, S., & Fenton, J. (2006). Cost-benefit analysis of biomass supply chains. *Biomass and Bioenergy*, 30(4), 299-315.

Smith, P., Martino, D., Cai, Z., Gwary, D., Janzen, H., & Kumar, P. (2007). Agriculture. In Climate Change 2007: Mitigation of Climate Change. Contribution of Working Group III to the Fourth Assessment Report of the IPCC. *Cambridge University Press*.

Tilman, D., Hill, J., & Lehman, C. (2006). **Carbon-negative biofuels from low-input high-diversity grassland biomass**. *Science*, 314(5805), 1598-1600.

Tilvikiene, V., & Malakauskiene, R. (2019). Utilization of agricultural waste biomass for energy purposes: A case study in Lithuania. *Renewable Energy*, 133, 1027-1033.

Tshikovhi, A., & Motaung, T. E. (2023). Technologies and innovations for biomass energy production. *Sustainability*, *15*(16), 12121.

Van Groenigen, J. W., Huygens, D., Boeckx, P., & Kuyper, T. W. (2011). The soil N cycle: Coupling processes, players, and ecosystem services. *Ecosystems*, 14(4), 593-606.

Zhang, N., Wang, M., & Wang, N. (2002). Precision agriculture—a worldwide overview. *Computers and Electronics in Agriculture*, 36(2-3), 113-132.

Zhang, Y. H. P. (2013). Next generation biorefineries: Sustainability and versatility of sugar-based biorefineries. *Energy Science & Engineering*, 1(1), 27-41.

Zhang, Q., Hu, J., Lee, D. J., & Chang, Y. (2019). Energy recovery from waste biomass: A review of recent developments in biomass pyrolysis. *Renewable and Sustainable Energy Reviews*, 119, 109550.

Weiland, P. (2010). Biogas production: Current state and perspectives. *Applied Microbiology and Biotechnology*, 85(4), 849-860.

A DNA-BASED CHARACTERISATION FOR DUS TESTS: MOLECULAR MARKERS

Didem Karalar[1], Mehmet Emin Caliskan[1]*

[1] *Department of Agricultural Genetic Engineering Nigde Ömer Halisdemir University, Faculty of Agricultural Sciences and Technologies*

didemkaralar@ohu.edu.tr

ABSTRACT

Plant variety protection was implemented initially by registration of seeds by the Distinctness, Uniformity, and Stability (DUS) testing criteria. Since the late 90s, discussion of DUS criteria inefficiency and searching for a novel DNA-based method has shown up. The inefficiency of DUS criteria with the time and cost needed for every step of field testing regarding the thousands of plant registrations yearly makes the process debated. Also defining, characterizing, and distinguishing plants solely based on phenotypic and biochemical standards are not reliable. Recent advancements in the biotechnology area have led to the registration system in another way. The International Union for the Protection of New Varieties of Plants (UPOV) has proposed the use of molecular markers to address these constraints. Contrary to traditional DUS tests, the application of molecular markers pointed to significant advancement. Some molecular markers such as Single Nucleotide Polymorphisms (SNP) and Simple Sequence Repeats (SSRs) provide greater precision and annihilate one of the main hesitations; what if the chosen marker is not related to phenotype? There are case studies so far in crops like corn, wheat, rice, and others that have shown better performance than standard DUS traits to distinguish and fit pedigree information. Despite these advancements, the adoption of molecular markers in DUS testing faces several challenges, including the need for standardized protocols and the development of robust marker-trait associations. Additionally, the cost and infrastructure requirements for these technologies pose significant barriers, particularly in developing countries. Continuous research and globalisation are needed to standardise the molecular markers, develop the databases, and increase the reliable use of molecular markers in DUS testing across different crops and regions. This review highlights an overview of current practices, perspective on molecular marker utilisations the need for further innovations in DUS testing.

Keywords: DUS test, molecular marker, UPOV, plant variety protection

INTRODUCTION

Variety registration is the registration of novel plant varieties which are the Distinct - characters (either morphologic or biochemical) that are not greatly influenced by the environment so the cultivar must be unique and not have been registered before, Uniform - level, and type of variation of individuals within the cultivar which variation should be commercially acceptable, and Stability - refers to a cultivar must remain true to its description when it is reproduced or propagated. DUS tests have long been critical, serving as the principal framework for plant variety protection and registration by the International Union for the Protection of New Plant Varieties (UPOV) internationally. DUS tests adapted for each plant variety by time. DUS testing has traditionally placed a heavy emphasis on morphological variables such as plant seed color and size, plant length, leaf color and shape, flower color, etc.(Yu & Chung, 2021), biochemical markers on some plants, and recently molecular markers have begun to integrate (UPOV, 2019a). For many years, phenotypic methods have offered a strong basis for distinguishing between plant varieties, especially in established crops like rice, wheat, and maize, where these characteristics are generally constant and well-understood.

As biotechnology has advanced new tools and approaches emerged that aid in fastening and easing plant breeding, and a new era started. In this way, breeding techniques have become increasingly sophisticated, and the limitations of relying solely on morphological traits have become more apparent. Trials are time-consuming, by increasing numbers and in some countries exceeding numbers of candidate varieties make the process drastically expensive and labor-intensive along with the problem of decreased variability and need for reference collections (Bernet et al., 2003; Gunjaca et al., 2008). The stated problems lead to inconsistencies, particularly when environmental factors influence the expression of the traits (Jamali et al., 2019). The distinctions between varieties become less evident as breeding programs push the genetic variety boundaries, it is becoming more difficult to differentiate based solely on visual features. In some crops such as soybeans and barley, it is especially obvious where phenotypic changes between varieties can be subtle and difficult to detect using typical DUS testing methods (Achard et al., 2020; Cockram et al., 2012).

Despite the undeniable advantages of the utilization of molecular markers in DUS tests, the fact remains that they have some limitations and disadvantages. First of all, the utilization of these markers requires special equipment and expertise and in developing countries where biotechnological developments are uneven and access to resources is limited, that is a concerning circumstance (Ribaut et al., 2010). On the other hand where there's another side of concerns already rising, dependence on proprietary technology and genetic data creates questions about the availability of these resources to all stakeholders involved in the agriculture industry and intellectual property rights (Blakeney, 2011). Accessibility in terms of biotechnological applications and genetic data, the width and depth of databases, and the density of studied plants also vary, and the scarcity and quality of studies that will help distinction of varieties that will cover the entire genome for each plant reduces access to markers that can be utilized in this way. These difficulties demonstrate the need for continued investment in infrastructure and studies to ensure the benefits of molecular marker-based DUS testing are realized on a global scale. A plethora of investigations that began with PCR advent such as RAPD markers (Kresovich et al., 1992), microsatellite markers (Rongwen et al., 1995), and AFLP markers (Lombard et al., 2000) in the case of plant variety distinction. By the time UPOV stated the need for a DNA-based method due to reliability, grand potential, and high resolution on distinctiveness, toward this demand two different strategies had been created and a subgroup of Technical and Legal Experts of Biochemical and Molecular Techniques (BMT Review Group) stepped in.

The evolution from a purely phenotypic to a molecular marker-based approach to DUS testing constitutes a significant advancement in plant variety protection. This shift is a proactive response to the evolving demands of contemporary agriculture as well as a reaction to the shortcomings of conventional approaches (De Riek, 2001). Molecular markers offer promise for plant variety protection and global food security as they continue to be refined and their applications expanded. Nonetheless, molecular markers have the potential to revolutionize plant variety protection, but there are several issues that need to be handled before this can be fully realized.

CURRENT PRACTICES IN TRADITIONAL DUS TESTING

Plant variety protection continues to be based on Distinction, Uniformity, and Stability (DUS) testing, which verifies that new plant varieties meet strict requirements prior to receiving legal protection and being made available for commercial release. DUS testing is based on the Protection of Breeders' Rights governed by the Convention of the International Union for the Protection of New Varieties of Plants (UPOV). Breeders' rights protection systems provide exclusive rights to owners of plant varieties on production, distribution, and marketing, and compensate for the cost of developing new varieties (Yu & Chung, 2021). UPOV offers a uniform framework for the protection and registration of new plant varieties that should meet some requirements; the variety should be original, distinguishable from other varieties, and stable in between generations (UPOV, 1991). A key component of this protection is the need for new varieties to be subject to DUS testing and accepted successfully before they can receive legal protection.

A new variety must be Distinct from others based on a specific phenotypic trait (including morphological, physiological, or biochemical traits). Uniformity is achieved by consistent expression of these traits, with variations allowed only within predefined limits. By observing the variety over multiple generations (at least two generations for DUS test trials) to ensure that its essential characteristics remain stable through repeated propagation, stability is confirmed (UPOV, 1991). Phenotypic traits such as plant length are vital in cereal crops like wheat for U (uniformity) and D (distinctness). To achieve the DUS standards varieties need to exhibit uniformity across various habitats. For example in the testing of ornamental plants, like roses, leaf shape and size play a crucial role in determining distinctiveness; in the case of vegetables, like tomatoes, fruit size and shape determine uniformity for instance, a new tomato variety must reliably yield fruit with a specific form, such round. In crops like wheat, the primary morphological characteristics that contribute to distinctness are spike length, awning, and grain color (Yu & Chung, 2021). For example a study done on durum wheat, to test the uniformity and stability of the new variety, variety distinction closely monitored key components of grain yield, such as grain protein content and grain yield per spike (Blanco et al., 2011). In the utilization and development of molecular markers to account for environmental heterogeneity and assure comprehensive evaluation, field trials are carried out in a variety of locations and growing seasons and these are labor and resource-intensive, high cost, and documenting and expertise are necessary (Akbari et al., 2024; Bonow et al., 2009; Gupta et al., 2022; Kresovich et al., 1992; Li et al., 2008; Thapa Magar et al., 2021).

DUS testing is conducted on various plant species, including rice, wheat, and maize, are assessed annually to ensure that food safety standards are met worldwide (Jamali et al., 2019) demonstrating the continuous progress in plant breeding and the introduction of new varieties for various agricultural and horticultural purposes. There are 338 testing guidelines, as of August 2024, to conduct DUS tests. The identification of phenotypic traits is limited by phenotype-based DUS testing due to environmental influences that complicate the determination process. The reliability of DUS testing can be compromised by varieties that appear uniform across different environmental conditions (Yang et al., 2021). Additionally, it can be difficult to identify closely related varieties due to potential inconsistencies and possible errors in variety registration, which is due to the subjective nature of morphological markers (Korir et al., 2013). Applicability of DUS tests has been restricted due to the cost of extensive field trials, vast numbers of new plant varieties to register annually, and complex processes, especially for developing countries and small breeding programs with limited labor and funding. Countries like China, India, and Brazil, which have vast agricultural industries, encounter considerable difficulties when it comes to DUS testing. Every year, China alone assesses thousands of new plant varieties; this puts tremendous strain on the DUS testing system to guarantee that every variety satisfies the required standards for stability, distinctness, and uniformity. It is revealed that in China, almost 6200 thousand novel varieties were registered in 2013, and that demonstrates the extent of the problem (Jamali et al., 2019; Tian et al., 2015).

Significant regulatory changes have recently been approved to simplify and improve the DUS testing process. Recent UPOV changes strongly emphasize the use of molecular markers in addition to traditional phenotype-based DUS tests to increase efficiency and sensitivity (Yang et al., 2021). Efforts by the UPOV community, particularly the Biochemical and Molecular Methods (BMT) subgroup, and IMMODUS (Integration of Molecular Data into DUS Testing) project have shown that incorporating molecular methods in addition to standard phenotypic assessments into DUS testing is a useful way to overcome these issues (UPOV, 2019b; UPOV, 2021).

Although the powerful application of molecular markers in various plants with objective and repeatable evaluations, integration into DUS tests has been slow. Existing regulations have not kept up with these developments and have not provided the training, infrastructure, and protocol adjustments needed. Anyhow DUS tests are evolving into a new era that includes genomic technologies, new sequencing technologies (such as NGS), improving of MAS (marker-assisted selection to ensure plant variety Distinctnes, uniformity and stability evaluations with the fastening of rapid screening of large populations (Torkamaneh et al., 2018; Xu & Crouch, 2008).

To reduce the cost and time of traditional testing methods, recent efforts aim to integrate genomic data into the DUS framework. DUS tests measurements, criteria, and tools need to be standardized, and global cooperation is very important to facilitate trade and protect plant breeder's rights. The UPOV Convention sets global standards for DUS testing and plant variety protection by requiring member countries to adopt national laws that conform to UPOV recommendations (V. et al., 2023). By incorporating genomic markers, recent changes aim to improve DUS testing procedures by reducing arbitrary assessments and increasing accuracy. DUS testing remains important to ensure the distinctness, uniformity, and stability of new varieties despite challenges such as high costs, subjective morphological indicators, environmental factors, and slow adoption of genomic techniques.

MOLECULAR MARKERS IN DUS TESTING

Molecular markers have begun to be considered as very important tools in Distinctness, Uniformity, and Stability (DUS) tests which might improve or replace phenotype-based DUS tests. The most commonly used molecular markers in this field are Simple Sequence Repeats (SSR), Amplified Fragment Length Polymorphisms (AFLP), and Single Nucleotide Polymorphisms (SNP). SSRs, in particular, are highly sought-after markers due to their codominant nature and high level of polymorphism and can distinguish even very close plant varieties (Gunjaca et al., 2008). In the case of SSR markers do not provide sufficient resolution to assess genetic diversity, AFLPs can help with the abundance of markers within the plant genome (Noli et al., 2008). SNPs are the most abundant type of variation in the genome due to accuracy and within-reach situations due to high throughput genotyping technologies they become more important in a variety of distinction and genetic studies like mapping (Sun et al., 2020). Over morphological markers and biochemical markers, molecular markers offer numerous advantages. Molecular markers demonstrate higher sensitivity on distinction even phenotype is affected by the environment (Yu & Chung, 2021). As mentioned, stability and reproducibility of molecular markers provide consistent results (Sammour, 2014). By facilitating the simultaneous analysis of numerous traits, they also increase efficiency by lowering the time and resources required for DUS testing (Smýkal et al., 2008).

Applications of molecular markers in important crops have proven successful. SSR markers have been used in maize to supplement morphological data. This has increased distinction resolution and consistency with pedigree information, allowing for more precise identification of various varieties (Gunjaca et al., 2008). Microsatellite markers have been successful in rice, increasing the reliability of variety identification by differentiating varieties that would otherwise be challenging to identify just by traditional DUS tests based on phenotypic assessment (Pourabed et al., 2015). Similar to this, in durum wheat, AFLP, and SSR markers have exceeded morphological markers in distinguishing closely related genotypes, making them extremely beneficial for DUS testing (Noli et al., 2008).

However, the use of molecular markers in DUS testing faces challenges, including insufficient correlation between markers and phenotypic features. Phenotypic variability due to outer factors might still exhibit in some varieties that appear uniform at the molecular level which is crucial for DUS assessments (Gilliland & Gensollen, 2010). The reliability of molecular markers is also greatly influenced by the availability of deeply studied plants and databases. A lack of data can lead to incorrect conclusions about similarities or differences among varieties and complicate the validation process. Nevertheless, standardized markers are essential. Such diversity in applications, marker selection, and data validation can produce incorrect and inconsistent results due to environmental interactions that influence phenotypic expression (Arens et al., 2010). One major concern is the phenotype-marker association caused by the nature of the traits. Generally, they are used the identify genetic variations at specific loci but somehow a number of phenotypic traits could have complex expressions that is either not well understood or difficult to determine by only one marker such as yield, plant vigor, height, etc. controlled by many genes and effect by environmental factors. Thus it is difficult to determine these traits with a single marker or multiple markers (Cockram et al., 2012).

Resources and cost management are the factors that molecular markers couldn't fully integrate into plant variety distinctions and widespread utilization. In developing countries need for biotechnological instruments, maintaining and expanding marker databases, and researcher numbers make it difficult to implement (Ribaut et al., 2010). Achieving homogeneity and stability in DUS testing requires a comprehensive method that takes into account genetic and phenotypic data, and as technologies evolve, these limitations must be taken into account to fully utilize molecular marker technologies.

FUTURE DIRECTIONS AND CLOSING REMARKS

DUS testings will be improved with the utilization of molecular markers in the testing of distinctness, uniformity, and stability and it become more relevant, accurate, and efficient. This has come about despite the fact that progress has been made because of the challenges posed by using and developing molecular markers and even more so attempting to integrate genetic data and phenotypic assessments. Novel developments such as next-generation sequencing (NGS) technology that aids in the development of dense genome-wide markers that enable high-throughput analysis that positively influences DUS testing are expected in the future. The need for sequencing using NGS has also changed the field of biology including any form of structural molecular biology, by the rapid, low-cost, high-throughput genetic analysis. This lays the foundation for the – enabling strategies that may be appropriate for marker development and application in plant breeding and DUS evaluation in the future (Torkamaneh et al., 2018).

It is expected that the use of these emerging technologies such as NGS in DUS testing of crops will spur further new molecular markers development that will help in data mining large datasets and detecting even minor differences among plant species. These technologies, according to Davey et al. (2011), offer views about possibilities to modernize genetic and breeding approaches like GWAS and genomic selection. There is also a need for infusion of current practices, technological advancements, and appropriate legislation to harmonize the application of molecular markers with DUS testing. Alongside the application of NGS, the creation of multi-omics approaches that merge metabolomics, transcriptomics, proteomics, and genomics will allow for other high-level data processing technologies to be applied to DUS testing in the future.

In order to make the most of the opportunities offered by modern molecular methods, international legislation should be adapted to incorporate these aspects within DUS testing frameworks. This will extend support to the cross-border trade while safeguarding plant breeding rights and plant varieties, and will also enhance the scientific and legal continuity of the domain. Future work should aim at the creation of bioinformatics applications that are more advanced in terms of processing NGS data, developing markers, and testing the associations between molecular markers and phenotypes. This will help overcome the disadvantages of DUS testing, including a stronger correlation between genotype and phenotype and the influence of the environment on phenotypic traits. Combining modern molecular markers and technologies with traditional phenotypic-based assessments may provide greater accuracy, efficiency, and global application in DUS testing in the future.

REFERENCES

Akbari, A., Bahmani, K., Kazan, M., Bilgin, Ö. F., Rahimi, J., Darbandi, A. I., Kafkas, N. E., & Kafkas, S. (2024). Analysis of Fennel Breeding Populations Based on Distinctness, Uniformity, and Stability (DUS) Testing via Morphological Descriptors and DNA Molecular Markers. *Genetic Resources and Crop Evolution*. https://doi.org/10.1007/s10722-024-01934-3

Arens, P., Mansilla, C., Deinum, D., Cavellini, L., Moretti, A., Rolland, S., van der Schoot, H., Calvache, D., Ponz, F., Collonnier, C., Mathis, R., Smilde, D., Caranta, C., & Vosman, B. (2010). Development and evaluation of robust molecular markers linked to disease resistance in tomato for distinctness, uniformity and stability testing. *Theoretical and Applied Genetics*, *120*(3), 655–664. https://doi.org/10.1007/s00122-009-1183-2

Bernet, G. P., Bramardi, S., Calvache, D., Carbonell, E. A., & Asins, M. J. (2003). Applicability of molecular markers in the context of protection of new varieties of cucumber. *Plant Breeding*, *122*(2), 146–152. https://doi.org/10.1046/j.1439-0523.2003.00838.x

Blakeney, M. (2011). Recent developments in intellectual property and power in the private sector related to food and agriculture. *Food Policy*, *36*, S109–S113. https://doi.org/10.1016/j.foodpol.2010.10.011

Blanco, A., Mangini, G., Giancaspro, A., Giove, S., Colasuonno, P., Simeone, R., Signorile, A., De Vita, P., Mastrangelo, A., Cattivelli, L., & Gadaleta, A. (2011). Relationships between grain protein content and grain yield components through quantitative trait locus analyses in a recombinant inbred line population derived from two elite durum wheat cultivars. *Molecular Breeding - MOL BREEDING*, *30*, 1–14. https://doi.org/10.1007/s11032-011-9600-z

Bonow, S., Von Pinho, E. V. R., Vieira, M. G. C., & Vosman, B. (2009). Microsatellite Markers in and around Rice Genes: Applications in Variety Identification and DUS Testing. *Crop Science*, *49*(3), 880–886. https://doi.org/10.2135/cropsci2008.06.0380

Cockram, J., Jones, H., Norris, C., & O'Sullivan, D. M. (2012). Evaluation of diagnostic molecular markers for DUS phenotypic assessment in the cereal crop, barley (Hordeum vulgare ssp. Vulgare L.). *Theoretical and Applied Genetics*, *125*(8), 1735–1749. https://doi.org/10.1007/s00122-012-1950-3

Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., & Blaxter, M. L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, *12*(7), 499–510. https://doi.org/10.1038/nrg3012

De Riek, J. (2001). ARE MOLECULAR MARKERS STRENGTHENING PLANT VARIETY REGISTRATION AND PROTECTION? *Acta Horticulturae*, *552*, 215–224. https://doi.org/10.17660/ActaHortic.2001.552.24

Gilliland, T. J., & Gensollen, V. (2010). Review of the Protocols Used for Assessment of DUS and VCU in Europe – Perspectives. In C. Huyghe (Ed.), *Sustainable use of Genetic Diversity in Forage and Turf Breeding* (pp. 261–275). Springer Netherlands. https://doi.org/10.1007/978-90-481-8706-5_37

Gunjaca, J., Buhinicek, I., Jukic, M., Sarcevic, H., Vragolovic, A., Kozic, Z., Jambrovic, A., & Pejic, I. (2008). Discriminating maize inbred lines using molecular and DUS data. *Euphytica*, *161*(1), 165–172. https://doi.org/10.1007/s10681-007-9518-z

Gupta, A. J., Anandhan, S., Manjunathagowda, D. C., Benke, A. P., Mahajan, V., Kad, S. K., & Singh, M. (2022). Complement test for distinctiveness, uniformity and stability testing of kharif onion (Allium cepa L.) varieties. *Genetic Resources and Crop Evolution*, *69*(6), 2217–2229. https://doi.org/10.1007/s10722-022-01372-z

Jamali, S. H., Cockram, J., & Hickey, L. T. (2019). Insights into deployment of DNA markers in plant variety protection and registration. *Theoretical and Applied Genetics*, *132*(7), 1911–1929. https://doi.org/10.1007/s00122-019-03348-7

Korir, N. K., Han, J., Shangguan, L., Wang, C., Kayesh, E., Zhang, Y., & Fang, J. (2013). Plant variety and cultivar identification: Advances and prospects. *Critical Reviews in Biotechnology*, *33*(2), 111–125. https://doi.org/10.3109/07388551.2012.675314

Kresovich, S., Williams, J. G. K., McFerson, J. R., Routman, E. J., & Schaal, B. A. (1992). Characterization of genetic identities and relationships of Brassica oleracea L. via a random amplified polymorphic DNA assay. *Theoretical and Applied Genetics*, *85*(2), 190–196. https://doi.org/10.1007/BF00222859

Li, L., Wang, J., Guo, Y., Jiang, F., Xu, Y., Wang, Y., Pan, H., Han, G., Li, R., & Li, S. (2008). Development of SSR markers from ESTs of gramineous species and their chromosome location on wheat. *Progress in Natural Science*, *18*(12), 1485–1490. https://doi.org/10.1016/j.pnsc.2008.05.012

Lombard, V., Baril, C. P., Dubreuil, P., Blouet, F., & Zhang, D. (2000). Genetic Relationships and Fingerprinting of Rapeseed Cultivars by AFLP: Consequences for Varietal Registration. *Crop Science*, *40*(5), 1417–1425. https://doi.org/10.2135/cropsci2000.4051417x

Noli, E., Teriaca, M., Sanguineti, M. C., & Conti, S. (2008). Utilization of SSR and AFLP markers for the assessment of distinctness in durum wheat. *Molecular Breeding*, *22*, 301–313. https://doi.org/10.1007/s11032-008-9176-4

Pourabed, E., Jazayeri Noushabadi, M. R., Jamali, S. H., Moheb Alipour, N., Zareyan, A., & Sadeghi, L. (2015). Identification and DUS Testing of Rice Varieties through Microsatellite Markers. *International Journal of Plant Genomics*, *2015*(1), 965073. https://doi.org/10.1155/2015/965073

Ribaut, J.-M., de Vicente, M. C., & Delannay, X. (2010). Molecular breeding in developing countries: Challenges and perspectives. *Current Opinion in Plant Biology*, *13*(2), 213–218. https://doi.org/10.1016/j.pbi.2009.12.011

Rongwen, J., Akkaya, M. S., Bhagwat, A. A., Lavi, U., & Cregan, P. B. (1995). The use of microsatellite DNA markers for soybean genotype identification. *Theoretical and Applied Genetics*, *90*(1), 43–48. https://doi.org/10.1007/BF00220994

Sammour, R. (2014). Cultivars identification based on biochemical markers. *Research & Reviews in BioSciences*, *8*, 347–358.

Smýkal, P., Horáček, J., Dostálová, R., & Hýbl, M. (2008). Variety discrimination in pea (Pisum sativum L.) by molecular, biochemical and morphological markers. *Journal of Applied Genetics*, *49*(2), 155–166. https://doi.org/10.1007/BF03195609

Sun, C., Dong, Z., Zhao, L., Ren, Y., Zhang, N., & Chen, F. (2020). The Wheat 660K SNP array demonstrates great potential for marker-assisted selection in polyploid wheat. *Plant Biotechnology Journal*, *18*(6), 1354–1360. https://doi.org/10.1111/pbi.13361

Thapa Magar, B., Acharya, S., Gyawali, B., Timilsena, K., Upadhayaya, J., & Shrestha, J. (2021). Genetic variability and trait association in maize (Zea mays L.) varieties for growth and yield traits. *Heliyon*, *7*. https://doi.org/10.1016/j.heliyon.2021.e07939

Tian, H.-L., Wang, F.-G., Zhao, J.-R., Yi, H.-M., Wang, L., Wang, R., Yang, Y., & Song, W. (2015). Development of maizeSNP3072, a high-throughput compatible SNP array, for DNA fingerprinting identification of Chinese maize varieties. *Molecular Breeding*, *35*(6), 136. https://doi.org/10.1007/s11032-015-0335-0

Torkamaneh, D., Boyle, B., & Belzile, F. (2018). Efficient genome-wide genotyping strategies and data integration in crop plants. *Theoretical and Applied Genetics*, *131*(3), 499–511. https://doi.org/10.1007/s00122-018-3056-z

UPOV, International Convention for the Protection of New Varieties of Plants of December 2, 1961, as Revised at Geneva on November 10, 1972, on October 23, 1978, and on March 19, 1991. Available online: https://www.upov.int/edocs/pubdocs/en/upov_pub_221.pdf

UPOV, International Union for the Protection of New Varieties of Plants (2013a) Guidance on the use of biochemical and molecular markers in the examination of distinctness, uniformity and stability (DUS), TGP/15. https://www.upov.int/edocs/tgpdocs/en/tgp_15.pdf. 26 Apr 2019 (2019a)

UPOV, International Union for the Protection of New Varieties of Plants "BMT Group Initiatives in Molecular Techniques." International Union for the Protection of New Varieties of Plants.(2019b).

UPOV, International Union for the Protection of New Varieties of Plants, "IMMODUS: Integration of Molecular Data into DUS Testing." International Union for the Protection of New Varieties of Plants.(2021).

V., S., Sandra, N., Ravishankar, K. V., & Chidambara, B. (2023). Molecular Techniques for Testing Genetic Purity and Seed Health. In M. Dadlani & D. K. Yadava (Eds.), *Seed Science and Technology: Biology, Production, Quality* (pp. 365–389). Springer Nature. https://doi.org/10.1007/978-981-19-5888-5_15

Xu, Y., & Crouch, J. H. (2008). Marker-Assisted Selection in Plant Breeding: From Publications to Practice. *Crop Science*, *48*(2), 391–407. https://doi.org/10.2135/cropsci2007.04.0191

Yang, C. J., Russell, J., Ramsay, L., Thomas, W., Powell, W., & Mackay, I. (2021). Overcoming barriers to the registration of new plant varieties under the DUS system. *Communications Biology*, *4*(1), 1–10. https://doi.org/10.1038/s42003-021-01840-9

Yu, J.-K., & Chung, Y.-S. (2021). Plant Variety Protection: Current Practices and Insights. *Genes*, *12*(8), Article 8. https://doi.org/10.3390/genes12081127

DIETARY EFFECTS OF ALGERIAN SODIUM BENTONITE ON GROWTH PERFORMANCE AND BIOCHEMICAL PARAMETERS IN BROILER CHICKENS

O. BESSEBOUA[1], A. AYAD[2], J.L. HORNICK[3], H. BENBAREK[4]

1 Department of Agronomic and Biotechnological Sciences, Faculty of Nature and Life Sciences, Univ. H. Ben Bouali, Chlef, Algeria
2 Department of Environment and Biological Sciences, Faculty of Nature and Life Sciences, Univ. of Bejaia, Algeria
3 Fundamental Applied Research and Animal Health (FARAH), Faculty of Veterinary Medicine, Univ. of Liège, Liège, Belgium
[4] Department of Agricultural Sciences, Faculty of Nature and Life Sciences, Univ. M. Stambouli, Mascara, Algeria
Corresponding author e-mail: benbarekh@gmail.com

ABSTRACT

The present experiment was conducted to investigate the effect of supplementing poultry feed with graded levels of *Algerian sodium bentonite* (Na-B) on growth performance and the development of villus height in jejunum and some biochemical parameters during 50 days in broiler chickens. A number of 420 one-day old broiler chicks (Arbor Acres) were obtained from a commercial hatchery. The birds were randomly allocated into six groups (A, B, C, D, E and F). The treatments were 0 (control), 1%, 2%, 3%, 4% and 5% of Algerian Na-B levels. The results obtained indicate clearly that weight gain in the chickens fed treatments containing 4% Na-B had greater weight gain than the chickens fed different treat- ments (0, 1%, 2%, 3% and 5% Na-B). Feed conversion rate (FCR) was lower birds supplemented with Na-B 4% (2.45) than control group (3.06). Maximum feed consumption was observed in the birds' control (5,655.3 g), while the lowest was noted in the chickens with diet added 4% Na-B (5,009.5 g) ($p< 0.05$). The weight of duodenum, jejunum and ileum was decreased for the Algerian Na-B supplemented group, compared with the control group. The villus height was affected by dietary treatments (1%, 2%, 3% and 5%) on days 18 and 50 ($p< 0.05$). Feeding the supplemented graded levels Na-B resulted in an increase in plasma cholesterol, triglyceride and HDL concentrations at 50 days of age, compared with the control group. These results showed clearly that the Na-B from Algeria can improve the growth performance in broiler chickens. Thus, dietary inclusion of Na-B had positive effect on plasma triglyceride, cholesterol and HDL values in broiler chickens at the end experiment.

Keywords: Algeria, clay, growth performance, feed supplementary, poultry.

INTRODUCTION

Clay is a natural economic substance and a highly abundant product in the nature. Bentonite, one main constituent of clays, is white, light weight rock deposit, composed mostly of salts of hydrated aluminosilicates of sodium (Na), potassium (K), calcium (Ca) and occasionally iron, magnesium, zinc, nickel, etc. The special properties of bentonite, such as hydration, swelling, water adsorption and viscosity, made it a valuable material for wide range of applications in industrial and farming systems (Miazzo *et al.,* 2000). Bentonite as a feed additive has been used successfully in poultry without any harmful effects (Prvulović *et al.,* 2008; Safaeikatouli *et al.,* 2010). The use of clay supplements in animal and poultry feed manufacturing is not new.

Several studies showed that poultry feed supplemented with so- dium bentonite (Na-B) can improve growth performance (Damiri *et al.,* 2010; Prvulović *et al.,* 2008; Safaeikatouli *et al.,* 2010; Salari *et al.,* 2006). Dietary clay supplements (bentonite and kaolinite) have been used as binding and lubricating agents in the production of pelleted feeds for chickens (Owen *et al.,* 2012).

Bentonite, as a toxin binder, de- creased the adverse effect of aflatoxin (Shi *et al.,* 2009) improved the performance (Pasha *et al.,* 2007) and reduced mycotoxin concentration in the livers of affected birds (Bailey *et al.,* 2006). In natural breeding farms, the consumption of soil is a natural phenomenon observed in all animal species. It is know that hens consume the soil fauna voluntarily or by eating earthworms and insects.

There are two types of naturally occurring bentonites: calcium and Na-B (Wright, 1968); there are clays originating from the smectites and their physical properties are those of this mineral group. Variations in interstitial water and exchangeable cations in interlayer of the different space affect the properties of bentonite and, thus, the commercial uses of the different types of bentonite (Adamis *et al.,* 2005). Indeed, Khanedar *et al.* (2012) showed that the use of either kinds of bentonite in diets at 1% could improve the broiler performances; however, addition of 1.5% bentonite had not any significant effect in this study. Today there is a variety of commercial clay, especially bentonite, available in the market in the form of animal feed additives with different chemical characteristics (Magnoli *et al.,* 2008).

In Algeria, the Na-B is abundant in the North West area (Mostaganem, Maghnia) and it is the most important deposit witha production of 20,000 tons per year (Ministry of Energy and Mines, 2015). To our knowledge, there are very few works published showing the effects of different levels of clay from Algeria on broiler performance. The present experiment was conducted to investigate the effect of supplementing poultry feed with graded levels of Algerian Na-B on growth performance and the development of villus height in jejunum and some biochemical parameters during 50 days in broiler chickens.

MATERIALS AND METHODS

Animals and dietary treatments

This study was conducted in Misserghin farm in Oran (western Algeria). The experimental protocol was approved by the Scientific Faculty Council of the University M. Istambouli (Mascara, Algeria).

A number of 420 one-day old broiler chicks (Arbor Acres) were obtained from a commercial hatchery.

The birds were randomly allocated into six groups (A, B, C, D, E and F; 70 birds/group) and housed in pens of identical size (2.35 × 6 m) in a deep litter system with a straw floor. The birds were fed a balanced commercial broiler ration *ad libitum* for a period of 7 weeks. Chicks were managed according to the guidelines suggested by Cobb Broiler Commercial Management Guide. Birds were vaccina- ted against Gumboro (IBDL, Ceva), Newcastle (HB1, Ceva) according to laboratory recommendations. In order to prevent coccidiosis, the chicks were treated by anticoccidial during 36 days (Salinomycine 200). The chicks were reared for 7 weeks maintaining all the hygienic measures in a well-ventilated poultry house.

The treatments were 0 (control), 1%, 2%, 3%, 4% and 5% of Algerian Na-B levels for starter (0 to 14 days), grower (15 to 28 days) and finisher (29 to 50 days) periods. Clay used in this experiment was grayish abundant in the area of study (Maghnia, Algeria). The chemical composition of Algerian Na-B is presented in *Table 1* (Debieche and Kaoua, 2014).

Growth performances

All birds were weighted individually after their arrival from the hatchery to the experimental farm (initial weight) and on day 50. Feed intake, body weight gain and feed conversion ratio (FCR) were calculated every 5 days. The daily weight gain (DWG) was calculated as follows: DWG (g/d) = (Final weight – Initial weight) / 50. The FCR used the following formula: FCR = Total feed consumption / Total final weight. Mortality was recorded daily for each pen.

Histomorphological and blood samples

During the experiment, after weighting, five chicks per treatment were randomly selected and killed by cervical dislocation for histomorphological analysis of the intestinal mucosa. The gastrointestinal tract was weighted after removal of the content. The tissue samples for histology were taken from the jejunum at days 18 and 50. The samples were fixed in 4% buffered formalin for 48 hrs. The processing consisted of serial dehydration, clearing, and impregnation with paraffin. Tissues sections, 5 µm thick were cut by a microtome and were fixed on slides. A staining procedure was carried out using hematoxylin and eosin. The slides were examined on an optical microscope.

Table 1 - Chemical composition (%) of sodium bentonite (Na-B) from Algeria (Maghnia, North West area)

Compositional profile	%
SiO_2	64.98
Al_2O_3	16.08
Fe_2O_3	2.93
CaO	0.61
MgO	3.51
K_2O	2.02
Na_2O	3.88
TiO_2	0.2
Loss on ignition	6.07

The images were analyzed using Neubauer ruling. Villus heights were measured according to the method described by Ritz *et al.* (1995).

Blood samples were collected randomly from brachial vein in tubes containing heparin at days 18, 29 and 50. The plasma obtained was centrifuged (1500 × g for 15 min) and stored at -20°C until measurement for cholesterol, triglycerides and high density lipoprotein (HDL) concentrations using a spectro-photometer reader, according to the manufacturer recommendation (Spinreact, S.A./S.A.U. Sant Esteve de Bas, Spain).

Data analysis

Statistical analyses were carried out in Statview (Version 4.55). Statistical analysis was performed using *t*-test to compare between different groups. The data were expressed as mean ± SE, and $P< 0.05$ was considered significant.

RESULTS AND DISCUSSION

Initial and final body weight (BW), weight gain, feed intake, FCR and mortality rate of the chickens fed different levels treatments are presented in *Table 2*. The initial body weight of chicks did not differ between the dietary treatments. At the end of the experiment (day 50), birds supplemented with graded levels of Algerian Na-B had a greater body weight, compared with control group (1,847.9 g). The birds supplemented with Na-B 4% had a higher body weight (2,044.1 g) than other groups. The average daily weight gain (from day 1 to 50) was higher for chicks supplemented with graded levels of Algerian Na-B (38.2 to 40.1 g) than for control group (36.4 g). The results obtained indicate clearly that weight gain in the chickens fed treatments containing 4% Na-B had greater weight gain than the chickens fed different treatments (1%, 2%, 3% and 5% Na-B).

Feed conversion rate (FCR) was lower in birds supplemented with Na-B 4% (2.45) than in control groups (3.06). In addition, Na-B supplemented birds (Group C) had a higher FCR than groups B, D, E and F (*Table 2*). Feed intake and increased weight gain in the chickens fed ration containing 4% Na-B recorded an improved FCR, in comparison with the control and all other diets treatment. Maximum feed intake was observed in the birds' control (5,655.3 g), while the lowest was noted in the chickens with diet added 4% Na-B (5,009.5 g). There is a significantly difference between the control and the chicken fed different treatments ($p< 0.05$). The mortality rate was lower for the birds fed supplemented with Na-B 4% (1.4%) than other groups. The groups that received the Na-B 1%, 2% and 3% showed higher mortality rate (8.6%, 7.1% and 12.9%, respectively) than the control group (4.3 %).

The means of the weight of intestinal part for dietary treatments are shown in *Table 3*. The weight of duodenum, jejunum and ileum was decreased for the Algerian Na-B supplemented group, compared with the control group. *Fig. 1* shows the means of jejunum villus height from broilers fed different diets. The villus height was affected by dietary treatments (1%, 2%, 3% and 5%) on days 18 and 50. However, birds fed supplemented Na-B 4% decreased the villus height, compared with the control group on day 18 ($p< 0.05$).

The effects of different dietary treatments on plasma lipids on broiler chickens are presented in *Table 4*. When compared with control group, feeding the supplemented graded levels Na-B resulted in increase in plasma cholesterol, triglyceride and HDL concentrations at 50 days of age.

Table 2 - Effect of graded levels of Algerian Na-B on the performance of broiler chickens

Item	Diets treatments (Sodium bentonite %, n=70)					
	0	1	2	3	4	5
Initial body weight (g)	41.08	41.14	40.31	40.31	40.25	40.87
Final body weight (g)	1847.88	1948.94	2035.75	2025.08	2044.06	1995.78
Daily weight gain (g)	36.14	38.16	39.91	39.70	40.08	39.10
Feed intake (g/bird)	2223.63[a]	1907.73[b]	2003.93[b]	1917.47[b]	1856.33[b]	1916.60[b]
Feed conversion rate (%)	2.12	1.66	1.75	1.72	1.79	1.94
Mortality rate (%)	4.28	8.57	7.14	12.85	1.42	5.71

[a,b] A significant difference in mean feed intake (g/bird) between the control group (0 Na-B level) and the treated groups (1%, 2%, 3%, 4% and 5% Na-B level) is indicated by letters (p < 0.05).

The present study shows that inclusion of Algerian Na-B in diets of broilers chickens improves weight gain and decreases the values of FCR (*Table 2*). In addition, the final body weight (day 50) of broilers containing different levels Algerian Na-B was higher than that of control group. The reason for this improvement may be the action of silicate minerals enhancing the digestibility of certain nutrients (Safaei Katouli *et al.*, 2012). Similar results have been described by Eser *et al.* (2011), who used the sepiolite in broiler diet.

Also, a higher growth response caused by bentonite supplemented diets has been reported in several animal species (Ibrahim *et al.*, 2000; Ivan *et al.*, 1992; Jacques *et al.*, 1986). However, the bentonite level above 4% could decrease the body weight gain in broiler chicken. A positive effect of bentonite on body gain seems to be inclusion dose on broiler dietary (Grosicki *et al.*, 2000). The feed efficiency of Algerian Na-B at different levels revealed non- significant difference statistically, but decreases the rate of FCR in broilers chicken, compared with control group. Bentonite at 4% level rather depressed the performance of chicks (Tauqir *et al.*, 2001). In the present experiment, we noticed that Algerian Na-B 5% in diet decrease the daily weight gain of broiler chicken, compared with all treated groups. Besides, additional Na-B level in feed higher 5% reduces the growth performance of birds (Mabbett, 2005). Damiri *et al.* (2012) reported that best results by addition low Na-B levels may be due to increased retention time of digesta in intestinal tract and more nutrients using. Therefore, the retention time increased had no negative effects on feed intake and increased performance.

Low weight gain and feed intake obtained in broiler with a Na-B level were in agreement with those reported by Tauqir and Nawaz (2001). It may be due to viscose nature of Algerian Na-B, which absorbs much water and decreased passage rate of digesta in lumen (Damiri *et al.*, 2012).

Table 3 - Effect of graded levels of Algerian Sodium bentonite (Na-B) on weight intestinal part of broiler chickens

Sodium bentonite (%)	Weight intestinal part (mean ± SE), g		
	Duodenum (n=5)	Jejunum (n=5)	Ileum (n=5)
0	17.97 ± 3.97^a	25.00 ± 6.78^a	23.20 ± 5.33^a
1	13.29 ± 1.49	21.06 ± 3.12	20.83 ± 6.61
2	12.20 ± 1.68	15.75 ± 2.86^b	13.94 ± 2.64^b
3	13.46 ± 2.10	19.94 ± 3.50	16.56 ± 3.00
4	11.52 ± 1.90^b	18.03 ± 3.20	15.41 ± 1.30^b
5	12.72 ± 2.50^b	18.10 ± 5.90^b	17.64 ± 2.50

Data presented in *Table 2* demonstrates that addition of the Algerian Na-B in feed had significant effect on the feed intake, compared with the control group ($p< 0.05$). Our results are different from of those obtained by Tauqir *et al.* (2001), who reported the use of level diet Na-B 2.5% improved feed consumption, compared with high Na-B level (>2.5%) that might have deleterious effect on the performance of birds.

However, Pasha *et al.* (2007) indicated that the indigenous Na-B 0.5% and 1% levels gave better results at the level of levels on birds' performance (Kubena *et al.,* 1993; Dale and Wyatt 1995).

Due to highly adhesive nature of the Na-B, it was suggested that Na-B absorbs moisture resist the flow of digesta through the gastrointestinal tract affecting negatively the feed intake (Van Olphen, 1963). This could be partially explained by the effect of clay on bile salts preservation (Prvulović *et al.,* 2007).The role of clinoptilolite, one of the clay, is involved in the adsorption and immobilization of the pathogenic flora and protection against their bile salt conjugation (Habold *et al.,* 2009).

In this study, the results of FCR were lower in birds receiving graded Na-B diets than those of the control group. The different levels of Na-B had clearly an effect on FCR, but the FCR was improved by adding in diet Na-B 4%. This is in accordance with the results of some studies showing that the use of Na-B in broiler chickens diet would improve their weight gain (Prvulović *et al.,* 2008; Tauqir *et al.,* 2001). Moreover, some researchers found that animal diets containing kaolin and zeolite have been shown an improvement body weight gain and FCR in chicken (Cabuk *et al.,* 2004; Hesham *et al.,* 2004; Incharoen *et al.,* 2009;Safaeikatouli *et al.,* 2010).

Table 4 - Effect of different levels of Algerian Sodium bentonite (Na-B) on broiler plasma lipids

	Plasma lipids (n=5, mean ± SE)		
Diets treatment	**Cholesterol (mg/dL)**	**Triglyceride (mg/dL)**	**HDL (mg/dL)**
0%			
Day 18	58.12 ± 31.96^{a}	48.76 ± 16.07	37.12 ± 19.53
Day 29	78.80 ± 33.03^{B}	63.81 ± 09.34	40.51 ± 09.31^{A,B}
Day 50	53.50 ± 28.68^{a}	37.67 ± 20.10	33.38 ± 11.36^{C,D}
1%			
Day 18	65.04 ± 12.41	42.73 ± 24.97^{a}	49.79 ± 09.24
Day 29	59.74 ± 14.80	41.02 ± 27.21	59.26 ± 22.61
Day 50	40.17 ± 22.49	55.12 ± 25.92	37.28 ± 08.90
2%			
Day 18	67.44 ± 20.52^{A}	96.37 ± 26.98^{a,b,A,B}	50.82 ± 24.21
Day 29	78.80 ± 33.03	57.30 ± 35.46^{A}	65.86 ± 16.81^{A}
Day 50	61.20 ± 26.61	76.74 ± 52.27^{B}	51.71 ± 10.70
3%			
Day 18	47.52 ± 09.82^{a,A}	67.58 ± 05.81	43.39 ± 07.66^{a}
Day 29	78.46 ± 10.79^{a,C}	49.21 ± 13.36	70.27 ± 06.67^{a,B}
Day 50	68.80 ± 03.15	43.26 ± 17.14	54.71 ± 10.70^{E}
4%			
Day 18	70.19 ± 29.87	50.55 ± 18.03	45.68 ± 13.95^{a}
Day 29	44.02 ± 23.43^{C}	14.88 ± 08.23	57.34 ± 24.58^{b}
Day 50	56.67 ± 39.58^{D}	45.58 ± 21.51	57.92 ± 14.90^{a,b,C,E}
5%			
Day 18	41.03 ± 29.54	45.74 ± 22.34^{b}	36.17 ± 19.57
Day 29	38.46 ± 08.22^{B}	30.79 ± 23.96	47.81 ± 06.31^{b}
Day 50	62.22 ± 26.47^{D}	53.12 ± 17.91	44.61 ± 11.24^{b,D}

^{a,b} Values with different superscripts in the same groups at different days that differ statically (p< 0.05).
^{A,B,C,D,E} Values with differences in the same day between different groups differ significantly (p< 0.05).

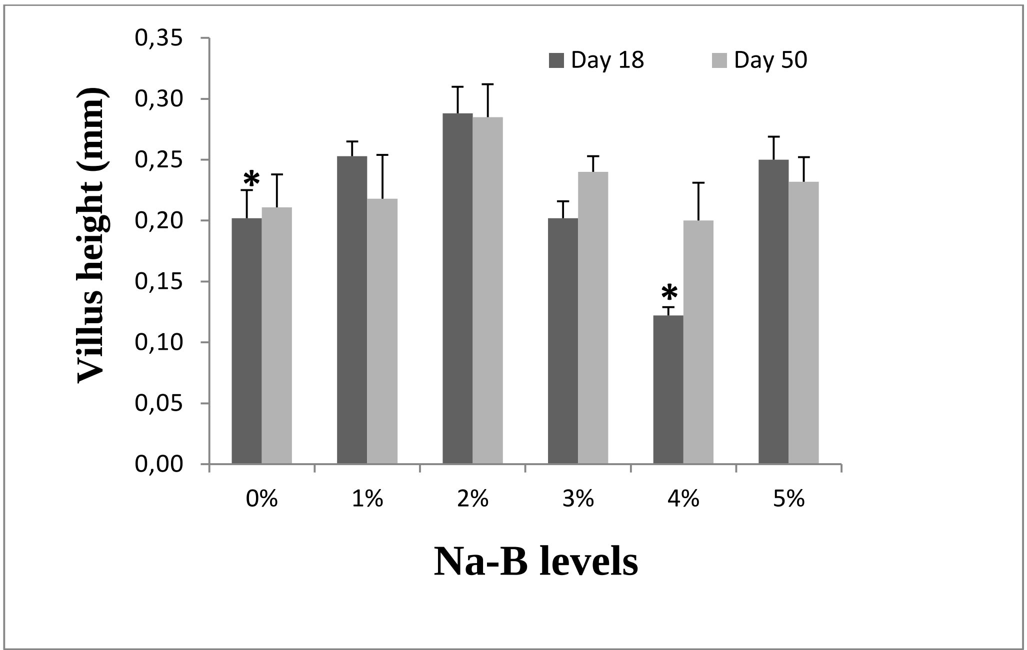


Figure 1 - Effect of different levels of Algeria Sodium bentonite (Na-B) on villus heights (mm) of broiler chickens (n=5). A significant difference in mean villus height between the Na-B level treated groups (0%, 1%, 2%, 3%, 4%and 5%) at Day 18 and Day 50 is indicated by asterisk (*)(*P* < 0.05).

Our results agree with the data obtained by Safaeikatouli *et al.* (2010), where the FCR was increased in broiler diet Na-B 3%, compared with kaolin 3% and Na-B 1.5% treatments. Also, Damiri *et al.* (2012) observed that addition of Na-B 3.75% in diet of broilers fed decreased feed intake and weight gain. Salari *et al.* (2006) indicated that chickens fed diets containing 1% and 2% of Na-B consumed more food. These latter had more weight gain and less FCR. Several studies have reported that weight gain in chicks given low energy diets is not affected by bentonite (Sellers *et al.,* 1980). Likewise, Tauqir *et al.* (2001) indicated that the interaction between diet Na-B and energy was found in broiler chickens.

According to Xu *et al.* (2003), a shortening of the villus and deeper crypts may lead to poor nutrient absorption, increased secretion in the gastrointestinal tract and lower performance. It has been suggested that greater villi height is an indicator that the function of intestinal villi is activated (Langhout *et al.*, 1999; Shamoto and Yamauchi 2000). In the present experiment, the histological aspect of the jejunum of broiler chickens gives orientation concerning the potential for using Na-B in broiler feed. The result of the present study indicates that supplementation of broiler with Algerian Na-B increased the villi height of jejunum at the end of experiment (*Fig.1*). The feed supplemented with graded Algerian Na-B had no effect statistically, but decreases slightly on the weight intestine parts, compared with the control group.

The results of this study correspond with those reported by Xia *et al.* (2004), who noted that the diet supplemented with montmorillonite increased the villus height and decreased crypt depth. Similarly, other study has reported that higher villus height were observed when the diet was treated with prebiotics and probiotics (Pelicano *et al.,* 2005). This could be explained by an increased epithelial cell turnover due to feeding of fed microbial (Awad *et al.,* 2008). According to Cera *et al.* (1988), maximum absorption and digestion capacity is given by a large luminal area with high villi and mature enterocytes, and is essential to animal development. As regards the weight of intestine parts, the present data showed a decrease for birds fed with Algerian Na-B, compared with the control group what demonstrate that the feed supplemented with different Algerian Na-B levels had no effect on the weight intestine of broilers (*Table 3*).

When compared with the control group, diets supplemented with different levels Na-B resulted in a remarkable increase in the plasma triglyceride, cholesterol and HDL concentrations. Kececi *et al.* (1998) have shown that some serum bioche- mical changes could be ameliorated by bentonite administration to the diet in broiler chickens. Habold *et al.* (2009) reported that the presence of clay in the intestinal lumen promotes the hydrolysis of triglycerides and increases the concentration of free fatty acids.

Decreased plasma cholesterol in chicks given Na-B in diet is consistent with the general reduction of lipo- genesis (Donaldson *et al.,* 1972) and impaired lipid transport (Tung *et al.,* 1972) in chicks and specific inhibition of hepatic cholesterol biosynthesis (Kato *et al.,* 1969).

CONCLUSIONS

The results obtained under the conditions of this experiment showed that the Algerian Na-B can improve the growth performance in broiler chickens. The inclusion of 4% Algerian Na-B in diets is more effective on the weight gain and feed intake. Also, supplementation of grade Algerian Na-B at 4% level was found beneficial in ameliorating the feed conversion rate in broiler chickens. Thus, dietary inclusion of Na-B had positive effect on plasma triglyceride, cholesterol and HDL values in broiler chickens at the end experiment.

REFERENCES

Adamis, Z., Fodor, J. & Williams, R.B. (2005). Bentonite, kaolin and selected Ccay minerals. World Health Organization, Geneva, Switzerland.

Awad, W., Ghareeb, K. & Böhm, J. (2008). Intestinal structure and function of broiler chickens on diets supplemented with a synbiotic containing *Enterococcus faecium* and oligosaccharides. *Int.J.Mol.Sci.,* 9(11): 2205-2216, DOI:10.3390/ ijms9112205

Bailey, C.A., Latimer, G.W., Barr, A.C., Wigle, W. L., Haq, A.U., Balthrop, J.E. & Kubena, L.F. (2006). Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. *J.App. Poult.Res*., 15(2): 198-206, DOI.org/ 10.1093/japr/15.2.198

Cabuk, M., Alcicek, A., Bozkurt, M. & Akkan, S. (2004). Effect of *Yucca schidigera* and natural zeolite on broiler performance. *Int.J.Poult.Sci*., 3(10): 651-654, DOI: 10.3923/ijps. 2004.651.654

Cera, K.R., Mahan, D.C., Cross, R.F., Reinhart, G.A. & Whitmoyer, R.E. (1988). Effect of age, weaning and posweaning diet on small intestinal growth and jejunal morphology in young swine. *J.Anim.Sci.,* 66(2): 574-584, DOI: 10.2527/jas1988. 662574x

Dale, N. & Wyatt, R.D. (1995). Impact of a sodium bentonite and an alumi- nosilicate on the protecting chicks from aflatoxicosis. *Poult.Sci.,* 74(1): 134.

Damiri, H., Chaji, M., Bojarpour, M., Eslami, M. & Mamoei, M. (2010). The effect of sodium bentonites on economic value of broiler chickens diet. *J.Anim.Vet.Adv.,* 9(20): 2668- 26670.

Damiri, H., Chaji, M., Bojarpour, M. & Mamoei, M. (2012). Effect of different sodium bentonite levels on performance, carcass traits and passage rate of broilers. *Pak.Vet.J.*, 32(2): 197-200.

Debieche, M. & Kaoua, F. (2014). Characterization and valorization of two Algerian bentonites in the waterproofing systems. *Mat.Sci. Appl.*, 5: 347-362, DOI: 10.4236/ msa.2014.55040

Donaldson, W.E., Tung, H.T. & Hamilton, P.B. (1972). Depression of fatty acid synthesis in chick liver (*Gallus domestic*us) by aflatoxin. *Comp.Bioch.Physiol.*, 4: 843-847.

Eser, H., Yalçin, S., Yalçin, S. & Şehu, A. (2011). Effects of sepiolite usage in broiler diets on performance, carcass traits and some blood parameters. *Kafkas Univ.Vet.Fak. Derg.*, 18(2): 313-318, DOI:10.9775/ kvfd.2011.5472

Grosicki, A. & Domańska, K. (2000). Use of bentonite to affect cadmium toxicokinetics. *Mengen-und Spurenelemente* 20, Arbeitstagung, 609-614.

Habold, C., Reichardt, F., Le Maho, Y., Angel, F., Liewig, N., Lignot, J.H. & Oudart, H. (2009). Clay ingestion enhances intestinal triacylglycerol hydrolysis and non-esterified fatty acid absorption. *Br.J.Nutr.*, 102: 249-257, DOI:10.1017/S0007114508190 274

Hesham, M.T., Hegazy, A.A. & Hussein, Y.A. (2004). Efficiency of kaolin and activated charcoal to reduce the toxicity of low level of aflatoxin in broilers. *J. King Saud Univ.-Sci.*, 5: 145-160.

Ibrahim, I.K., Shareef, A.M. & Al- Joubory, K.M.T.(2000). Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody forma- tion in broiler chickens during aflatoxicosis. *Res.Vet.Sci.*, 69(2): 119-122, DOI: 10.1053/rvsc.2000.0390

Incharoen, T., Khambualai, O. & Yamauchi, K. (2009). Performance and histological changes of the intestinal villi in chickens fed dietary natural zeolite including plant extract. *Asian J.Poult.Sci.*, 3(2): 42-50, DOI: 10.3923/ajpsaj.2009.42.50

Ivan, M., Dayrell, M. de S. & Hidiroglou, M. (1992). Effects of bentonite and monensin on selected elements in the stomach and liver of fauna-free and faunated sheep. *J. Dairy Sci.*, 75: 201-208, DOI: 10.3168/jds. S0022-0302(92)77754-6

Jacques, K.A., Axe, D.E., Harris, T.R., Harmon, D.L., Bolsen, K.K. & Johnson, D.E. (1986). Effect of sodium bicarbonate and sodium bentonite on digestion, solid and liquid flow, and ruminal fermentation characteristics of forage sorghum silage-based diets fed to steers. *J.Anim.Sci.*, 63(3): 923-932, DOI: 10.2527/jas1986.633923x

Kato, R., Onoda, K. & Omori, Y. (1969). Effect of aflatoxin B1 on the incorporation of 14 C-acetate into cholesterol by rat liver. *Experimentia*, 25(10): 1026.

Kececi, T., Oguz, H., Kurtoglu, V. & Demet, O.(1998). Effects of polyvinylpolypyrrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. *Br.Poult.Sci.*, 39(3):452-458, DOI: 10.1080/0007166988 9051

Khanedar, F., Vakili, R. & Zakizadeh, S. (2012). Effects of two kinds of bentonite on the performance, blood biochemical parameters, carcass characteristics and tibia ash of broiler chicks. *Glob.Vet.*, 9(6): 720-725, DOI: 10.5829/idosi.gv.2012.9.6. 61151

Kubena, L.F., Harvey, R.B., Phillips,T.D. & Clement, B.A. (1993). Effect of hydrated sodium calcium aluminosilicates on the aflatoxicosis in the broiler chicks. *Poult.Sci.*, 72(4): 651-657, DOI: 10.3382/ps. 0720651

Langhout, D.J., Schutte, J.B., Van Leeuwen, P., Wiebenga, J. & Tamminga, S. (1999). Effect of dietary high and low-methylated citrus pectin on the activity of the ileal microflora and morphology of the small intestinal wall of broiler chickens. *Br.Poult.Sci*., 40(3): 340–347, DOI: 10.1080/00071669987421

Mabbett, T. (2005). Integrated manage- ment of mycotoxins. *Poult.Int*., 44(8): 10-14.

Magnoli, A.P., Tallone, L., Rosa, C.A.R., Dalcero, A.M., Chiacchiera, S.M. & Torres Sánchez, R.M. (2008). Commercial bentonites as detoxifier of broiler feed contaminated with aflatoxin. *Appl. Clay Sci*., 40(1-4): 63-71, DOI: 10.1016/j.clay.2007.07.007

Miazzo, R., Rosa, C.A.R., De Queiroz Carvalho, E.C., Magnoli, C., Chiacchiera, S.M., Palacio, G.,Saenz, M., Kikot, A., Basaldella, E. & Dalcero, A. (2000). Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks. *Poult.Sci.*, 79(1): 1-6, DOI: 10.1093/ps/79.1.1

Ministry of Energy and Mines (Algeria). http://www.mem-algeria.org/francais/ index.php?page=la-bentonite.Accessed 07 April 2015.

Owen, O.J., Nodu, M.B., Dike, U.A. & Ideozu, H.M. (2012). The effects of dietary kaolin (clay) as feed additive on the growth performance of broiler chickens. *Greener J.Agric.Sci*., 2(6): 233-236.

Pasha, T.N., Farooq, M.U., Khattak, F.M., Jabbar, M.A. & Khan, A.D. (2007). Effectiveness of sodium bentonite and two commercial products as aflatoxin absorbents in diets for broiler chickens. *Anim. Feed Sci.Tech.*, 132(1-2): 103-110, DOI: 10.1016/j.anifeedsci.2006.03. 014

Pelicano, E.R.L., Souza, P.A., Souza, H.B.A., Figueiredo, D.F., Boiago, M.M., Carvalho, S.R. & Bordon, V.F. (2005). Intestinal mucosa development in broiler chickens fed natural growth promoters. *Braz.J.Poult.Sci.*, 7(4): 221- 229, DOI: 10.1590/S1516-635X2005000 400005

Prvulović, D., Jovanović-Galović, G.A., Stanić, B., Popović, M. & Grubor- Lajšić, G. (2007). Effects of a clinoptilolite supplement in pig diets on performance and serum parameters. *Czech J.Anim.Sci.*, 52(6): 159-164.

Prvulović, D., Kogić, D., Grubor-Lajšić, G.G. & Kosarcić, S. (2008). The effects of dietary inclusion of hydrated aluminosilicate on performance and biochemical parameters of broiler chickens. *Turk.J.Vet.Anim.Sci*., 32: 183-189.

Ritz, C.W., Hulet, R.M., Self, B.B. & Denbow, D.M. (1995). Growth and intestinal morphology of male turkeys as influenced by dietary supplementation of amylase and xylanase. *Poult.Sci.*, 74(8): 1329- 1334, DOI: 10.3382/ps.0741329

Safaei Katouli, M., Boldaji, F., Dastar, B. & Hassani, S. (2010). Effect of different levels of kaolin, bentonite and zeolite on broilers performance. *J.Biol.Sci.*, 10(1): 58-62.

Safaei Katouli, M., Boldaji, F., Dastar, B. & Hassani, S. (2012). Growth response and tibia bone characteristics in broilers fed diets containing kaolin, bentonite and zeolite. *J. Anim. Feed Sci.*, 21: 334- 344.

Salari, S., Kermanshahi, H. & Nasiri Moghaddam, H. (2006). Effect of sodium bentonite and comparison of pellet vs mash on performance of broiler chickens. *Int.J.Poult.Sci.*, 5(1): 31-34, DOI: 10.3923/ijps.2006. 31.34

Sellers, R.S., Harris, G.C. & Waldroup, P.W. (1980). The effect of various dietary clays and fillers on the performance of broilers and laying hens. *Poult.Sci.*, 59(8): 1901-1906, DOI.org/10.3382/ps.0591901

Shamoto, K. & Yamauchi, K. (2000). Recovery responses of chick intestinal villus morphology to different refeeding procedures. *Poult.Sci.*,79(5): 718-723, DOI: 10. 1093/ps/79.5.718

Shi, Y., Xu, Z., Sun, Y., Wang, C. & Feng, J. (2009). Effects of two different types of montmorillonite on growth performance and serum profiles of broiler chicks during aflatoxicosis. *Turk.J.Vet.Anim.Sci.,* 33: 15-20.

Tauqir, N.A., Sultan, G.I. & Nawaz, H. (2001). Effect of different levels of bentonite with varying energy levels on the performance of broilers. *Int.J.Agric.Biol.,* 3: 85-88.

Tauqir, N.A. & Nawaz, H. (2001). Performance and economics of broiler chicks fed on rations supplemented with different levels of sodium bentonite. *Int. J. Agric. Biol.,* 3: 149-150.

Tung, H.T., Donaldson, W.E. & Hamilton, P.B. (1972). Altered lipid transport during aflatoxicosis. *Toxicol.Appl.Pharmacol*., 22(1): 97-104, DOI: 10.1016/0041-008X(72) 90229-3

Van Olphen, H. (1963). An introduction to clay colloid chemistry. *Interscience Publishers,* New York, DOI: 10.1002/ jps.2600530238

Wright, P.C. (1968). The meandu creek bentonite - a reply. *J.Geol.Soc.Aust.*, 15(2): 347-350, DOI: 10.1080/00167 616808728702

Xia, M.S., Hu, C.H. & Xu, Z.R. (2004). Effects of copper-bearing montmorillonite on growth perfor- mance, digestive enzyme activities, and intestinal microflora and morphology of male broilers. *Poult.Sci.,* 83(11): 1868-1875, DOI: 10.1093/ps/83.11.1868

Xu, Z.R., Hu, C.H., Xia, M.S., Zhan, X.A. & Wang, M.Q. (2003). Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. *Poult.Sci.,* 82(6): 1030- 1036, DOI: 10.1093/ps/82.6.1030.

EFFECT OF SACCHAROMYCES CEREVISIAE FEED SUPPLEMENTATION ON HAEMATOLOGY AND REPRODUCTIVE PARAMETERS FOR ALGERIAN RABBITS

Omar BESSEBOUA[1,], Abdelhanine AYAD[2]*

[1] *Department of Agronomic and Biotechnological Sciences, Faculty of Nature and Life Sciences, University H.Benbouali, 02000, Chlef, Algeria*

[2] *Department of Environment and Biological Sciences, Faculty of Nature and Life Sciences, University of Bejaia, 06000, Bejaia, Algeria*

Corresponding author e-mail: besseboua.omar@gmail.com

ABSTRACT

This study aims at investigating the effect of *Saccharomyces cerevisiae* (SC) supplementation on reproductive performance, haematological parameters and fertility of rabbits under Algerian conditions. The animals were divided into three groups and received the same feed ration during the experimental period. The control group received a basal diet without feed additives (Group#0) and the two yeast SC groups received 0.3 and 0.6 g/day per head (Group#1 and Group#2, respectively). Semen and blood samples were collected for determination of semen parameters and haematology. The weights of rabbits treated with SC 0.3 g/day were statistically significantly different ($P< 0.05$) from the control groups and group treated with SC 0.6 g/day. There were significant differences between the treatment groups for (RBCs), haemoglobin (HGB), haematocrit (HCT) and mean corpuscular haemoglobin (MCH) values, with higher values in rabbits supplemented with SC 0.3 g/day and 0.6 g/day, compared to those in the control group. The scrotal diameter did not differ between the dietary treatments. When compared with the control group, feeding rabbits graded levels of SC resulted in an increase in the average semen volume, mass motility and individual motility at day 51 of the experiment. On the other hand, the sperm concentration was significantly lower ($P< 0.05$) in rabbits supplemented with SC 0.3 g/day and 0.6 g/day during the two months compared to that in the control group. The spermatozoa mortality rate was lower for the rabbits supplemented with SC 0.3 g/day and 0.6 g/day (15.7% and 11.4%, respectively), compared to that in the control group (24%). In conclusion, this study has shown that inclusion of SC 0.3 g/day and 0.6 g/day in the diets of rabbit has positive effects on body weight and sperm analysis. Moreover, it increases the level (RBCs), haemoglobin (HGB), haematocrit (HCT) and mean corpuscular haemoglobin (MCH).

Keywords: *Saccharomyces cerevisiae*, feed, haematological parameters, sperm, rabbits.

INTRODUCTION

It is known that high levels ofantibiotics have been used in food- producing animals as growth promoters and for disease prophylaxis.However, there is a major problem among human consumers due to theoccurrence of antibiotic residues in meat because of the wide use ofantibiotics and antibiotic-resistantbacteria in animals. Therefore, theEuropean Union

Commission banned the use of antibiotics as a growth enhancer in the diets of animals (Castanon, 2007). It is therefore imperative to replace the overuse ofantibiotics and to search for a new safealternative for health improvement andinfection control in animals.

The probiotics (bacterial and yeastcultures) are non-pathogenic microbialadjuncts, which have been used as feedsupplements and also as growthpromoters, improving the immune system of animals by promoting thecomposition and microbial balance in its gut. Several studies have garnered attention over the years on the use of probiotics as alternative feed additives in order to replace antibiotics and synthetic chemical feed supplements (Higginbotham and Bath, 1993; Brydt*et al.,* 1995; Sumeghy, 1995; Strzetelski, 1996). Yeasts are being widely used in food, medicine and the cosmetic industry due to their bioactive and nutritional components, such as peptides, amino acids, beta-glucan, glutathione, cerebroside and zinc (Cha *et al.,* 2004, 2008; Kinosita *et al.,* 2007;Lee *et al.,* 2005).

Moreover, it increases the levelof some haematological parameters, such as RBCs, HGB, HCT, and GCTs.There has been scientific interest over the last two decades in the supplement *Saccharomyces cerevisiae* (SC), whichincreases the cellulolytic rumenbacteria (Ogunade *et al.*, 2019). Pinheiro *et al.* (2020) and Arif *et al.* (2020) reported that the strains of SC have potential as probiotics and are adsorbent of aflatoxin B1. The Algerianrabbit represents a significant portion of the agricultural economy. The statistical data revealed that more and more breeders are interested in rabbit farms where the production of rabbit meat is the first activity (Saidj *et al.,* 2013). Rabbit meat is an important source of protein, rich in precious nutrients (essentially amino acids and lipids and low in fat content and cholesterol (< 59 mg/100 g) (Combes and Dalle Zotte, 2005). In the last years, the Algerian Department ofAgriculture has adopted a policy of diversifying animal production by encouraging rabbit farmers to invest more. Therefore, this research study was conducted to explore the impact ofSC supplementation on some reproductive parameters and haematological balance in male rabbitsunder Algerian conditions. It is known that high levels of antibiotics have been used in food-producing animalsas growth promoters and for diseaseprophylaxis.

MATERIALS AND METHODS

With regard to the ethical aspects,this experimental procedure was approved by the Scientific Committee at the University of Chlef (Report of Faculty Scientific Council #04 dated 30 September 2015).

Animal groups and feeding

The experiment was conducted in an experimental farm of the Department of Agronomic and Biotechnological Sciences(36°10' N, 1°19' E; University of Chlef, Algeria). This study was conducted from1 March 2016 to 30 April 2016. A total of nine rabbits aged 10 months were used for this study. The rabbits were in a good condition during the experimental period. The animals were divided into three groups and received the same feed ration during the experimental period. Before the beginning of the feeding experiment, the pre-trial period consisted of an adaptation period of two weeks for animals who werefed SC supplementation. Then, the rabbits were followed over six weeks, during which the collection and analysis of sperm were performed.

The animals were divided into three groups and received the same feed ration during the experimental period. The control group received a basal diet without feed additives (Group#0), and the two yeast SC groups received 0.3 g/day and 0.6 g/day per head (Group#1 and Group#2, respectively). The supplemented SC (CNCM I-1077, Lallemand Animal Nutrition) is marketed by the Vetam Company (Mostaganem, Algeria) and contains 20 × 10^9 CFU/g of live yeast. The water was distributed *ad libitum* for the study period. The rabbits were weighted at the start of the experiment and then on the following days 1, 15, 29, 44 and 51. Weight gain (WG) was calculated as follows: DWG (g/day) = (Final weight – Initial weight).

Blood and semen sampling

Whole blood samples were withdrawn from the marginal vein into EDTA tubes for haematological analysis. Haematological indices, such as red blood cells (RBCs), platelets (PLTs), haemoglobin (HGB), white blood cells (WBCs), lymphocytes (LCTs), monocytes (MCTs), granulocytes (GCTs), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red blood cell distribution index (RBDI), were measured. All these measurements were performed using an automated haematology analyser (SWELAB alfa, Boule Medical AB, Spanga, Sweden).

The semen was collected on days 21, 35, and 42 to analyse the various parameters of spermatozoa. The collected semen was transported quickly to the laboratory and kept at body temperature. The study protocols of the semen preparation and analysis were done according to Wyrobek and Bruce (1975). The volume of semen was simply measured using the graduations on the collection tube. The pH was measured with a digital pH meter. Sperm motility was estimated after semen collection by examining a small drop of semen placed on a warm slide and examined by light microscope. Sperm concentration was determined using a Thoma cell counting chamber. The determination of live and dead sperm cells was analysed using the eosin-nigrosin staining procedure according to Esteso *et al.* (2006).

Scrotal diameter was measured at the widest point of the scrotum with a graduated tape on days 14 and 45. Sexual activity (libido) was estimated by the time between introduction of the female into the male's cage and ejaculation. At the end of the experiment, the sexual behaviour of rabbit bucks was determined. A sexually receptive female was then dropped from one side of the chamber and allowed 30 min of stimulation (Sanna et al., 2015; Cicero et al., 2001). Mounting latency (ML) was estimated as time from female introduction into the cage to the occurrence of first mount. The intromission latency was defined as the duration interval between the introduction of the female and intromission by the male (Dabhakar and Zade, 2013; Pare et al., 2014; Mutwedu et al., 2019).

Statistical analyses

Statistical analyses of the results were carried out in Statview (Version 4.55). Statistical analysis was performed using t-tests to compare between different groups. The data were expressed as mean ± SE and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The final body weight of the rabbits fed different levels of treatment are presented in Table 1. The weights of rabbits treated with SC 0.3 g/day were significantly decreased (P< 0.05), compared to the group control and group treated with SC 0.6 g/day.

At the end of the experiment (day 51), the rabbits supplemented with graduated levels of SC had a slightly lower body weight than the control group (3.282 kg). Also, the rabbits supplemented with SC 0.3 g/day showed a continuous increase in body weight, as compared to the other groups.

The mean ± SE values of the blood parameters of treated and non- treated rabbits is shown in *Table 2*. There were significant differences between the treated groups for RBCs, HGB, HCT and MCH values, with higher values in rabbits supplemented with SC 0.3 g/day and 0.6 g/day, compared to the control group. PLT, WBCs and GCTs for the control group were higher than the treated groups. On the other hand, MCTs and LCTs were low in the control group compared to those in the treated groups, which was statistically insignificant (*P*> 0.05). Rabbits supplemented with SC showed slightly higher values in h haematological parameters, such as MCV and RBDI, compared to those reported for the control group.

The effect of SC supplementation on the scrotal diameter, sexual behaviour and sperm analysis of the rabbits is illustrated in *Table 3*. The scrotal diameter did not differ between the dietary treatments. The rabbits fed rations containing 0.3 g/day and

0.6 g/day SC recorded high intromission latency values in comparison to the control group. However, there was a significant difference in sexual behaviour between the controls and rabbits fed different levels of SC supplementation (*P*> 0.05).

Table 1 - Effect of graded levels of *Saccharomyces cerevisiae* (SC)on body weight in rabbits

	Diet treatment (kg)		
	Group#0 (n=3, ± SE)	**Group#3 (n=3, ± SE)**	**Group#6 (n=3, ± SE)**
Day 1	3.335 ± 0.23^{b}	2.305 ± 0.15^{a}	3.130 ± 0.25^{b}
Day 15	3.303 ± 0.25^{b}	2.305 ± 0.17^{a}	3.025 ± 0.26^{b}
Day29	3.278 ± 0.31^{b}	2.382 ± 0.19^{a}	3.027 ± 0.25^{b}
Day 44	3.302 ± 0.33^{b}	2.388 ± 0.19^{a}	2.992 ± 0.23^{b}
Day 51	3.282 ± 0.27^{b}	2.407 ± 0.22^{a}	3.027 ± 0.26^{b}

Table 2 - The impact of Saccharomyces cerevisiae (SC) on the haematological parameters of rabbits on different days

	Diet treatment		
	Group#0 (n=3, ± SE)	**Group#3 (n=3, ± SE)**	**Group#6 (n=3, ± SE)**
RBCs ($10^6/mm^3$)	$5.21 \pm 0.25^{a,b}$	6.29 ± 0.3^{a}	6.09 ± 0.22^{b}
HGB (g/dl)	$9.5 \pm 0.71^{a,b}$	12.9 ± 0.58^{a}	12.6 ± 0.25^{b}
PLT ($10^3/mm^3$)	352 ± 106.7	329.7 ± 85.6	281 ± 83..1
HCT (%)	$28.95 \pm 1.48^{a,b}$	36.13 ± 1.43^{a}	35.97 ± 1.1^{b}
WBCs ($10^3/mm^3$)	13.7 ± 1.13	9.9 ± 1.61	9.9 ± 3.26
LCTs ($10^3/mm^3$)	3.75 ± 1.34	5.3 ± 0.87	4.2 ± 1.01
MCTs ($10^6/mm^3$)	0.65 ± 0.21	1.33 ± 0.47	0.67 ± 0.31
GCTs ($10^6/mm^3$)	9.3 ± 2.26^{a}	3.27 ± 0.47^{a}	5.07 ± 2.94
MCV (%)	53.3 ± 3.25	57.53 ± 0.6	59.17 ± 1.62
MCH (pg)	$18.35 \pm 0.07^{a,b}$	20.53 ± 0.15^{a}	20.7 ± 0.79^{b}
MCHC (g/dl)	37.65 ± 2.33	35.73 ± 0.55	35.1 ± 0.6
RBDI (%)	14.85 ± 2.05	14.87 ± 0.40	15.67 ± 0.78

Red blood cells (RBCs), haemoglobin (HGB), platelets (PLT), haematocrit (HCT), white blood cells (WBCs), lymphocytes (LCTs), monocytes (MCTs), granulocyte (GCTs), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution index (RBDI).
[a,b] Values by the same letters between the treated groups on different days are statistically different in the same row ($P < 0.05$).

The pH of semen seems to be stable in rabbits supplemented with SCand in the control group, ranging from 7.33 to 8.00. When compared with control the group, rabbits fed diets with graded levels of SC (Group#1 and Group#2) had increases in mean semen volume, mass motility and individual motility at day 51 of the experiment. On the other hand, the sperm concentration was significantly lower ($P < 0.05$) in rabbits fed SC supplementation at 0.3 g/day and 0.6 g/day over the two months of the experiment compared to the control group. The spermatozoa mortality rate was lower for the rabbits fed SCsupplementation at 0.3 g/day and 0.6 g/day (15.7% and 11.4%, respectively), compared to that in the control group(24%).

Table 3 - Effect of graded levels of SC on scrotal diameter, sexual behaviour and sperm analysis in rabbits

	Diet treatment		
	Group#0 (n=3, ± SE)	**Group#3 (n=3, ± SE)**	**Group#6 (n=3, ± SE)**
Scrotal diameter (mm)			
Day 15	5.00 ± 0.30	5.67 ± 1.03	4.82 ± 0.72
Day 45	5.45 ± 0.60	5.98 ± 0.71	5.55 ± 1.14
Sexual behavior			
Mounting latency (Sec)	5.0 ± 3.0	5.0 ± 2.8	11 ± 2.83
Intromission latency (Sec)	5.33 ± 4.5	13.00 ± 2.8	21.7 ±18.5
Sperm analysis			
pH			
Day 21	7.33 ± 0.06	7.33 ± 0.58	7.50 ± 0.50
Day 51	8.00 ± 0.00	7.47 ± 0.50	8.00 ±0.00
Semen volume (ml)			
Day 21	0.67 ± 0.06	0.53 ± 0.12	0.43 ± 0.15
Day 51	0.40 ± 0.00	0.47 ± 0.29	0.67 ± 0.00
Mass motility			
Day 21	2.67 ± 1.53*	4.33 ± 0.58	6.00 ± 1.00*
Day 51	5.00 ± 0.00	5.33 ± 3.51	6.67 ± 0.58
Individual motility			
Day 21	2.00 ±1.73	1.67 ± 1.15*	4.00 ± 0.00*
Day 51	2.00 ± 0.00	2.67 ± 1.53	4.00 ± 0.00
Sperm concentration (10^6/ml)			
Day 21	269.3 ± 122.5*	52.9 ± 38.9*	206.3 ± 173.6
Day 51	390.0 ± 0.0	148.3 ± 51.3	259.4 ± 81.1
Mortality of spermatozoid (%)			
Day 21	43.3 ± 15.3	39.3 ± 4.6	25.3 ± 13.0
Day 51	24.0 ± 0.0	15.7 ± 9.1	11.0 ± 6.9

Values by asterisks between the treated groups on different days are statistically different in the same row (P< 0.05).

The present study was to investigate the effects of inclusion of the yeast SC in the diet on the haematological and sperm parameters of rabbits. According to the results of this study, the body weight gain of rabbits was not significantly affected by dietary SC supplementation. Thisconfirmed the previous findings of several studies, which noted no significant differences in the growth performance of rabbits fed a ration containing SC supplementation (Kimsé*et al.*, 2008; Brümmer *et al.*, 2010; Darwish *et al.*, 2011; Seyidoglu *et al.*, 2013; Seyidoglu and Galip, 2014; Belhassen *et al.*, 2016). Kimsé *et al.* (2012) noted that yeast did not affect final body weight, daily weight gain orfeed intake in New Zealand rabbits.Another study reported that the administration of SC to mice showed an insignificant improvement in growth performance (Abdel-Aziz *et al.*,2010). On the one hand, these differentresults may be due to the dose or kind of yeast used, strain of broilers, basal diet, and environmental conditions or refer to the mechanisms of growth promotion of yeast culture in rabbits, whereas generally, the positive relationship between SC and animal performance characteristics have been reported by several authors (Omat *et al.,* 2010; Saied *et al.,* 2011; Abou El- Naga *et al.,* 2012; Ezma *et al.,* 2012; Onwurah and Okejim, 2014, Mohamed*et al.,* 2015). On the other hand, Onifade and Babatunde (1996)reported that a diet supplemented with SC at 6.0 g/kg improved the feed quality in broiler chicks. Likewise, Waché *et al.* (2006) observed that the addition of SC can enhance diet and protein digestibility, which revealed better growth and food efficiency with yeast supplements. Moreover, Mohammadi *et al.* (2016) confirmed that using a 2% concentration of yeast in the diet improved growth and food utilization in three spots cichlid(*Cichlasoma trimaculatum*). Thedivergence of yeast results on weight gain could be due to the age of the rabbit, livestock conditions, type of feed, and type and dose of yeastincorporated into the food (Akpa *et al.*,2012).

Data are presented in *Table 2* anddemonstrate that the RBCs, HGB,HCT, GCT and MCV were significantlydifferent in rabbits receiving graded SC diets as compared to the controlgroup. This is in divergence with the previous results revealing that the use of SC in the diets of rabbits had no effect on some haematological parameters (Seyidoglu and Galip, 2014; Belhassen *et al.,* 2016). Seyidoglu *et al.* (2013) revealed no significant variations in hematological parameters in treatment groups, although they noted a slight rise in hematocrit and hemoglobin concentrations in rabbits supplemented with yeast. Other findings are supported by researchers where the values of HGB, HCT, RBCs, MCV, MCH and MCHC were unchanged in broiler chicks fed diets containing probiotics (Gheisari *et al.,* 2008; Shareef *et al.,* 2009; Saied *et al.,*2011). Moreover, Onifade *et al.* (1999)noted that HCT, HGB, MCV and MCH rose significantly (P< 0.05) in rabbits receiving SC supplementation. In another investigation, Paryad *et al.* (2008) observed that the addition ofboth 1.5% and 2% SC yeast significantly elevated WBCs and decreased the LCT ratios of chicks. Moreover, Mulatu *et al.* (2019) revealed that WBCs, packed cell volume (PCV) and HGB were higherin chickens fed a diet containing SC.In addition, Saied *et al.* (2011) showedthat dietary yeast has no effects on the PCV values of broiler chickens. However, Elghandour *et al.* (2019) observed that yeast-fed rabbits had more WBCs and LCTs, compared to the rabbited fed the control diet. It is noted that the LCT content may be a signal of improvement of humoral immune responses in rabbits fed diets supplemented with SC. Also, a relationship between dietary levels of SC and hematological parameters has been reported, and it could be a promoter of supplemental yeast in rabbits (Onifade *et al.,* 1999). Likewise, our results show that SC does not affect animal health. This was demonstrated by Krzysztof *et al.* (2012), who observed that probiotics have immunostimulatory effects.

The results of our present study were in agreement with those of Sharawy *et al.* (2015), who reported a non-significant increase in testicular volume in the probiotics group, compared to that in the non-treated groups, while other researchers noted that scrotal circumference in rams was significantly greater in the probiotics fed group than in the control group (Fernandez *et al*., 2004; Kerban, 2008). Moreover, Kheradmand *et al.* (2006) indicated that both the testicular size and scrotal circumference of rams were influenced by alimentation, and the scrotal circumference was greater in the treated groups than in the control group. This result can be explained by testicular growth being associated with the nutritional value of food. The present study revealed significant differences in the sperm analysis, namely, mass motility, individual sperm motility and sperm concentration (P< 0.05) and were similar to those seen in rams (Sharawy *et al.,* 2015). This result is in accordance with Emmanuel *et al.* (2019) and Helal *et al.* (2018), who observed remarkable enhancement in the sperm concentration and motility of rabbit bucks, which were supplemented with SC. These results are in agreement with results obtained previously, which recorded that the ingestion of probiotics might be recommended to improve sperm motility in human males (Valcarce *et al.*, 2017). The antioxidant activity of SC may be the key to improvement in the sperm parameters (Uskova and Kravchenko, 2009; Spyropoulos *et al.*, 2011; Ewuola, 2013; Mymrin *et al.*, 2017; Shehu *et al.*, 2016). It has been reported that yeasts and their extracts are sources of natural antioxidant cmpounds (Nishino and Ishikawa, 1998; Gazi *et al.*, 2001).

Our results are similar to those obtained by Helal *et al.* (2018), who reported insignificant differences in dead spermatozoa in the experimental groups (P> 0.05), while the lowest proportions of dead spermatozoa were recorded in the SC groups. Nevertheless, studies claim that compounds with antioxidant properties, such as SC, have the potential to inhibit oxidative damage in the cell membranes of sperm cells and fragmentation of sperm DNA caused by free radicals (Castellini, 2008; Mourvaki *et al.*, 2010). Our study showed an insignificant difference (P> 0.05) in semen pH values in rabbits fed dietary inclusions of SC. However, Emmanuel *et al.* (2019) showed a significant reduction in the pH of rabbits on a SC diet. Then, the pH in spermatozoa plays a principal role in regulating sperm motility and fertility competence (Holm *et al.*, 1998).

Generally, both mount and intromission latencies were used as indicators of sexual motivation (Dabhakar and Zade, 2013; Nchegang *et al.*, 2016). The effect of the addition of SC on male sexual capability measured by sexual behaviours, such as mounting and intromission latencies, were insignificant (P> 0.05), compared to controls. Helal *et al.* (2018) showed identical observations, with SC having a non-significant effect on the libido of rabbits. In another investigation, the mount and intromission latencies of the *Monsonia angustifolia* extract-treated groups showed insignificant differences compared to the control group (Gerda Fouche *et al.*, 2015).

CONCLUSIONS

The results of the present study showed that inclusion of SC in the diets of rabbits containing 0.3 g/day and 0.6 g/day have positive effects on the body weight and semen analysis of rabbits. Moreover, it increases the levels of some haematological parameters, such as RBCs, HGB, HCT and GCTs. More investigations are desired to document the benefits of dietary inclusion of SC required in rabbits under diverse environmental conditions using an appropriate number of animal experimental units.

REFERENCES

Ahmed, M.E., Abbas, T.E., Abdlhag, M.A. & Mukhtar, D.E. (2015). Effect of dietary yeast (*Saccharomyces cerevisiae*) supplementation on performance, carcass characteristics and some metabolic responses of broilers. *Anim. Vet. Sci*., 3(5): 5-10, DOI: 10.11648/j.avs.s.2015030501.12

Arif, M., Iram, A., Bhutta, M.A.K., Naiel, M.A.E., Abd El-Hack, M.E., Othman, S.I...& Taha, A.E. (2020). The biodegradation role of *Saccharomyces cerevisiae* against harmful effects of mycotoxin contaminated diets on broiler performance, immunity status, and carcasscharacteristics. *Animals*,10(2): 238, DOI: 10.3390/ani10020238

Belhassen, T., Bonai, A., Gerencsér, Z., Matics, Z., Tuboly, T., Bergaoui, R. & Kovacs, M. (2016). Effect of diet supplementation with live yeast *Saccharomyces cerevisiae* on growthperformance, caecal ecosystem and health of growing rabbits. *World Rabbit Sci.,* 24(3): 191, DOI: 10.4995/wrs.2016.3991

Castanon, J.I.R. (2007). History of the useof antibiotic as growth promoters in European poultry feeds. *Poultry Sci.,* 86(11): 2466-2471, DOI: 10.3382/ps.2007-00249

Cha, J.-Y., Heo, J.-S., Park, B.-K. & Cho, Y.-S. (2008). Effect of zinc-enriched yeast supplementation on serum zincand testosterone concentrations in ethanol feeding rats. *J. Life Sci.,* 18(7): 947-951, DOI: 10.5352/jls.20 08.18.7.947

Elghandour, M.M.Y., Tan, Z.L., Abu Hafsa, S.H., Adegbeye, M.J., Greiner, R., Ugbogu, E.A. …& Salem,A.Z.M. (2019).*Saccharomyces cerevisiaeas* aprobiotic feed additive to non and pseudo-ruminant feeding: a review. *J.Appl. Microbiol.,* 128(3): 658-674, DOI: 10.1111/jam.14416

Emmanuel, D., Amaka, A., Okezie, E., Sunday, U. & Ethelbert, O. (2019). Epididymal sperm characteristics,testicular morphometric traits andgrowth parameters of rabbit bucks fed dietary *Saccharomycescerevisiae* and/or zinc oxide. *Rev. Bras. Cienc. Avic.,* 21(1), DOI: 10.1590/1806-9061-2018-0803

Esteso, M.C. (2006). Functional significance of the sperm head morphometric size and shape for determining freezability in Iberian Red Deer (*Cervus elaphus hispanicus*) epididymal sperm samples. *J. Androl.,* 27(5): 662-670, DOI: 10.2164/jandrol.106.000489

Ezema, C.&Eze, D.C. (2010). Determination of the effect of probiotic (*Saccharomyces cerevisiae*)on growth performance and hematological parameters of rabbits. *Com. Clin. Path.,* 21(1): 73-76, DOI: 10.1007/s00580-010-1066-6

Fernández, M., Giráldez, F., Frutos, P., Lavín , P. & Mantecón, A. (2004). Effect of undegradable protein supply on testicular size, spermiogram parameters and sexual behavior ofmature Assaf rams. *Theriogenology,* 62(1-2): 299-310, DOI: 10.1016/j.theriogenology.2003.10.003

Fouche, G., Afolayan, A.J., Wintola,O.A., Khorombi, T.E. & Senabe, J. (2015). Effect of the aqueous extract of the aerial parts of *Monsonia angustifolia* E. Mey. Ex A. Rich., on the sexual behaviour of male Wistar rats. *BMC Complement. Altern. Med.,*15(1): DOI: 10.1186/s12906-015-0880-4

France, 29-30 November (2005). Abstracts of the 11èmes Journées de la Recherche Cunicole. *World Rabbit Sci.,* 14(sp): 35-50, DOI:10.4995/wrs.2006.536

Helal, F., El- Badawi, A., Abou-ward, G., El-Naggar, S., Hassan, A., Basyoney, M. & Morad, A. (2018). Semen quality parameters of adult male NZW rabbits fed diets added with two different types of probiotics. *Egypt. J. Nutr. Feeds,* 21(1): 125-132, DOI: 10.21608/ejnf.2018.75423

Higginbotham, G.E. & Bath, D.L. (1993). Evaluation of *Lactobacillus* fermentation cultures in calf feeding systems. *J. Dairy Sci.,* 76(2): 615-620, DOI: 10.3168/jds.s0022-0302(93)77382-8

Holm, L. & Wishart, G.J. (1998). The effect of pH on the motility of spermatozoa from chicken, turkey and quail. *Anim. Reprod. Sci.,* 54(1): 45-54, DOI: 10.1016/s0378-4320 (98)00142-0

Kimsé, M., Bayourthe, C., Monteils, V., Fortun-Lamothe, L., Cauquil, L., Combes, S. & Gidenne, T. (2012). Live yeast stability in rabbit digestive tract: Consequences on the caecalecosystem, digestion, growth anddigestive health. *Anim. Feed Sci. Technol.,* 173(3-4): 235-243, DOI: 10.1016/j.anifeedsci.2012.01.012

Kimsé, M., Bayourthe, C., Monteils, V., Fortun-Lamothe, L., Cauquil, L., Combes, S. & Gidenne, T. (2012). Live yeast stability in rabbit digestive tract: Consequences on the caecal ecosystem, digestion, growth and digestive health. *Anim. Feed Sci. Technol.*, 173(3-4): 235-243. DOI: 10.1016/j.anifeedsci.2012.01.012

Kinoshita, M., Hori, N., Aida, K., Sugawara, T. & Ohnishi, M. (2007). Prevention of melaninformation by yeast cerebroside in B16 mouse melanoma cells. *J. Oleo Sci.*, 56(12): 645-648, DOI:10.5650/jos.56.645

Lee, S., Kim, W.-G., Kim, E., Ryoo, I.-J., Lee, H. K., Kim, J. N. … & Yoo, I.-D. (2005). Synthesis and melanin biosynthesis inhibitory activity of (±)- terrein produced by *Penicillium sp.* 20135. *Bioorg. Med. Chem. Lett.*, 15(2): 471-473, DOI: 10.1016/j.bmcl. 2004.10.057

Mourvaki, E., Cardinali, R., Dal Bosco, A., Corazzi, L. & Castellini, C.(2010). Effects of flaxseed dietary

supplementation on sperm quality and on lipid composition of sperm subfractions and prostatic granules in rabbit. *Theriogenology*, 73(5): 629-637, DOI: 10.1016/j.theriogenology. 2009.10.019

Mulatu, K., Ameha, N. & Girma, M. (2019). Effects of feeding different levels of baker's yeast on performance and hematological parameters in broiler chickens. *J. World's Poult. Res.*, 9(2): 38-49, DOI: 10.36380/jw pr.2019.5

Mutwedu, V.B., Ayagirwe, R.B.B.,Bacigale, S.B., Mwema, L.M., Butseme, S., Kashosi, T. &Nyongesa, A.W. (2019). Effect of dietary inclusion of small quantities of *Mucuna pruriens* seed meal on sexual behavior, semen characteristics, and biochemical parameters in rabbit bucks (*Oryctolagus cuniculus*). *Trop. Anim. Health Prod.*, 51(5): 1195-1202, DOI: 10.1007/s11250-019-01808-2

Nada, S.A., Amra, H.A., Abdel-Aziz, K.B., Omara, E.A., Farag, I.M., Tawfek, N.S. & Darwish, H.R. (2011). *Saccharomyces cereviciae* potentially inhibit ochratoxicosis in male albino mice. *Toxicol. Lett.*, 205: S143-S144, DOI: 10.1016/j.toxlet. 2011.05.509

Nchegang, B., Mezui, C., Longo, F., Nkwengoua, Z.E., Amang, A.P. &Tan, P.V. (2016). Effects of the aqueous extract of *Eremomastax speciosa* (Acanthaceae) on sexual behavior in normal male rats. *BioMed. Res. Int.*, 1-10, DOI: 10.1155/2016/9706429

Ogunade, I.M., Lay, J., Andries, K.,McManus, C.J. & Bebe, F. (2019). Effects of live yeast on differential genetic and functional attributes of rumen microbiota in beef cattle. *J. Anim. Sci. Biotechnol.*, 10(1), DOI: 10.1186/s40104-019-0378-x

Onifade, A.A. & Babatunde, G.M. (1996). Supplemental value of dried yeast in a high-fibre diet for broiler chicks. *Anim. Feed Sci. Technol.*, 62(2-4): 91-96, DOI: 10.1016/s0377-8401 (96)00991-1

Onifade, A., Obiyan, R., Onipede, E., Adejumo, D., Abu, O…& Babatunde, G. (1999). Assessment of the effects of supplementing rabbit diets with a culture of *Saccharomyces cerevisiae* using growth performance, blood composition and clinical enzyme activities. *Anim. Feed Sci. Technol.*, 77(1-2): 25-32, DOI: 10.1016/s0377-8401(98)00244-2

Pinheiro, R.E.E., Rodrigues, A.M.D., Lima, C.E., Santos, J.T.O., Pereyra,C.M., Torres, A. M…& Muratori, M.C.S. (2020). *Saccharomyces cerevisiae* as a probiotic agent and a possible aflatoxin B1 adsorbent in simulated fish intestinal tract conditions. *Arq. Bras. Med. Vet. Zootec.*, 72(3): 862-870, DOI: 10.1590/1678-4162-11280

Saied, J.M., Al-Jabary, Q.H. & Thalij, K.M. (2011). Effect of dietary supplement yeast culture on production performance and hematological parameters in broiler chicks. *Int. J. Poult. Sci.*, 10(5): 376- 380, DOI: 10.3923/ijps.2011.376.380

Sanna, F., Contini, A., Melis, M.R. & Argiolas, A. (2015). Role of dopamine D4 receptors in copulatory behavior: Studies with selective D4agonists and antagonists in male rats. *Pharmacol. Biochem. Behav.,* 137, 110-118, DOI: 10.1016/j.pbb. 2015.08.012

Seyidoğlu, N., Galip, N. & Sonat, F.A. (2014). Effect of yeast culture on growth performance, haematological and biochemical indices of New Zealand white rabbits. *Uludağ Üniv. Vet. Fak. Derg.,* 32(2): 11-18, DOI: 10.30782/uluvfd.163480

Seyidoğlu, N. & Galip, N. (2014). Effects of *Saccharomyces cerevisiae* and *Spirulina platensis* on growth performances and biochemical parameters in rabbits. *Kafkas Üniv. Vet. Fak. Derg.,* 20(3): 331-336, DOI:10.9775/kvfd.2013.9988

Shehu, B.M., Ayanwale, B.A., Ayo, J.O.& Uchendu, C. (2016). Short communication: Effect of *Saccharomyces cerevisiae* supplementation on some biomarkers of oxidative stress in weaned rabbits during the hot-dry season. *World Rabbit Sci.,* 24(1): 67-70, DOI: 10.4995/wrs.2016.1656

Spyropoulos, B.G., Misiakos, E.P., Fotiadis, C. & Stoidis, C.N. (2010). Antioxidant properties of probiotics and their protective effects in the pathogenesis of radiation-induced enteritis and colitis. *Dig. Dis. Sci.,* 56(2): 285-294, DOI: 10.1007/s10 620-010-1307-1

Valcarce, D.G., Genovés, S., Riesco, M.F., Martorell, P., Herráez, M.P., Ramón, D. & Robles, V. (2017). Probiotic administration improves sperm quality in asthenozoospermic human donors. *Benef. Microbes*, 8(2): 193-206, DOI: 10.3920/bm20 16.0122

Waché, Y., Auffray, F., Gatesoupe, F.-J., Zambonino, J., Gayet, V., Labbé, L.& Quentel, C. (2006). Cross effects of the strain of dietary *Saccharomyces cerevisiae* and rearing conditions on the onset of intestinal microbiota and digestive enzymes in rainbow trout, *Onchorhynchus mykiss*, fry. *Aquaculture*, 258(1-4): 470-478, DOI: 1016/j.aquaculture.2006.04. 002

EVALUATION OF MARE'S CONFORMATION PARAMETERS AND ITS RELATIONSHIP WITH PREGNANCY RATE

Amel NAJJAR [1] and Emna ARARI [1]

[1] *Department of Animal Sciences, National Institute of Agronomy of Tunisia, University of Carthage, Tunisia.*

Corresponding author e-mail: amel.najarbenmaatou@inat.ucar.tn

ABSTRACT

The objective of the study was to determine mare's conformation parameters and their pregnancy rate. Thirty (30) Arabian and twenty (20) Thoroughbred mares were used in this study, and their conformation parameters were determined. The mare's weight was calculated from the chest circumference which was noted using a horse specific tape measure. The mare's vulva conformation was studied by the determination of the Caslick index. ANOVA showed that chest circumference and the weigh varied according to the breed, age and status of mares ($p<0.05$). However, the Caslick index varied only according to the breed ($p<0.01$), and it was lower in Thoroughbred mares compared to the Arabian mares (129±20 *vs* 147±32). The results showed that the pregnancy rate was higher in Thoroughbred mares compared to the Arabian ones ($p<0.01$). According to these findings, the conformation parameters were improved in Thoroughbred mares and which explains their higher pregnancy rate compared to the Arabian mares. These differences could be due to the breed's genetic traits.

Keywords: mares, conformation, chest circumference, Caslick index, pregnancy rate.

INTRODUCTION

Conformation parameters play a crucial role in determining the overall health and reproductive success of a mare. The evaluation of mare's conformation can provide valuable insight into her ability to carry a pregnancy and successfully a deliver healthy foal. Mares with poor conformation may have a lower pregnancy rate and be at a higher risk for reproductive issues. The objective of the study was to determine mare's conformation parameters and their pregnancy rate.

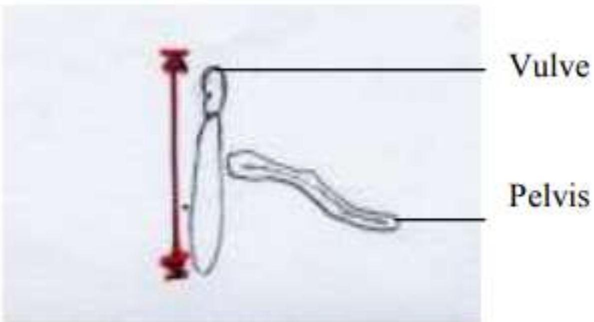
MATERIEL AND METHODS

The study took place at a private stud farm during the breeding season, from 01 February to 31 May. The stud farm is located in the delegation of Borj El-Amri, in the north of Tunisia, 25 km from Tunis. The area has an average annual rainfall of 456 mm and an average annual temperature of 17.8 °C.

Thirty (30) Arabian and twenty (20) Thoroughbred mares were used in this study, and their conformation parameters were determined. The mare's weight was calculated from the chest circumference which was noted using a horse specific tape measure (Merck & Co tape) according to the Reavell method (Reavell, 1999). The reading is taken directly from the tape measure when it was in place and properly adjusted.
The conformation of the mare's vulva was recorded using the Caslick index (Figure 1). This involves determining the length of the vulva between the pelvic floor and the dorsal commissure. The angle of inclination to the vulva was determined using a protractor (Papa et al., 2014).

Caslick index = Length of vulva (L) X angle of vulva (a) (Caslick, 1937)

If the index value obtained is less than or equal to 100, the vulval conformation is normal and the mare's fertility is considered good. If the index value is greater than 100, the vulval conformation is poor and the mare's fertility is considered low (AFDA, 2018).



Normal vulva conformation

Abnormal vulva conformation

Figure 1. Comparaison between normal and abnorla vulva conformation.

ANOVA was carried out using SAS software. Proc GLM was used to study the effect of breed, age and status of mares on conformation parameters. The DUNCAN test was performed to compare the means between groups.

RESULTS AND DISCUSSION

The results showed that chest circumference varied according to the breed, age and status of mares ($p<0.05$, Figures 2, 3 and 4). The weight was higher in Thoroughbred mares compared to the Arabian ones (574 kg vs 504 kg, $p<0.001$). The Thoroughbred mares are characterized by a greater growth potential than Arabian mares. This difference leads to inter-breed precocity. The Caslick index varied only according to the breed ($p<0.01$), and it was lower in Thoroughbred mares compared to the Arabian mares (129±20 *vs* 147±32). Thus, It can be concluded that Thoroughbred mares have better vulva conformation compared the Arabian mares. However, The findings of Talluri et al (2015) showed that the Caslick index was affected by the age and parity of the mare.

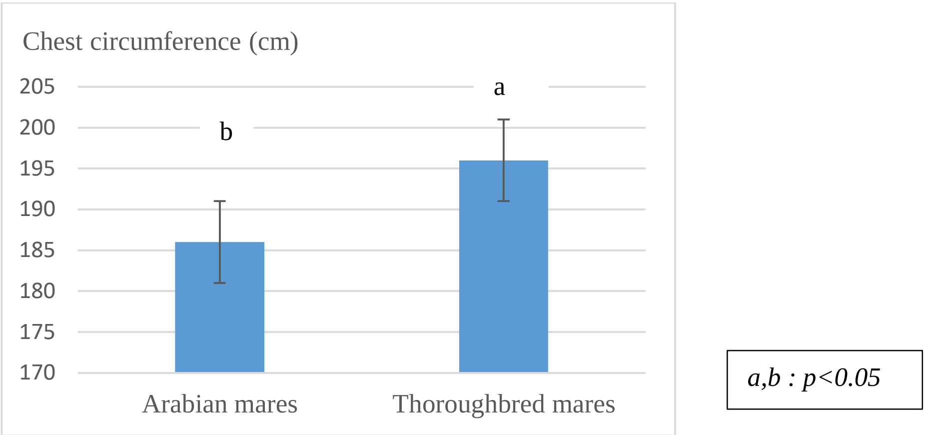


Figure 2. Variation of the mare’s chest circumference according to the breed (Means±SD).

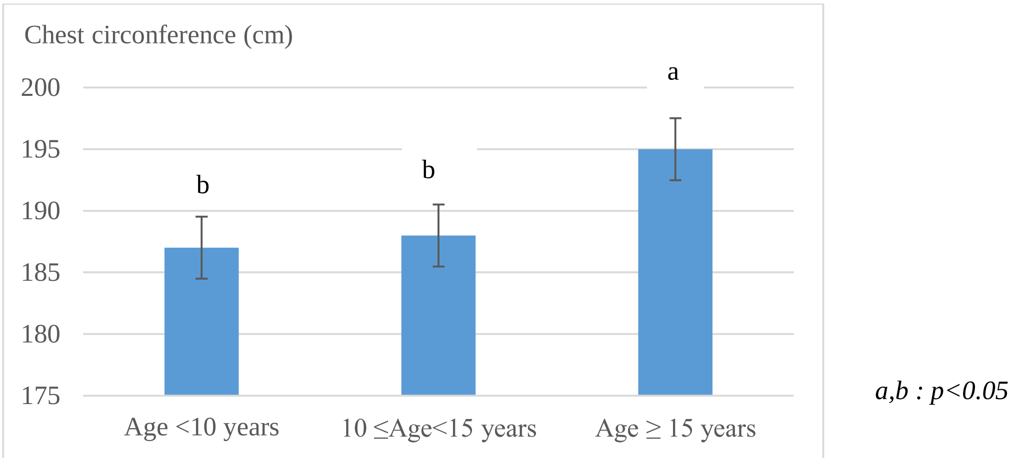
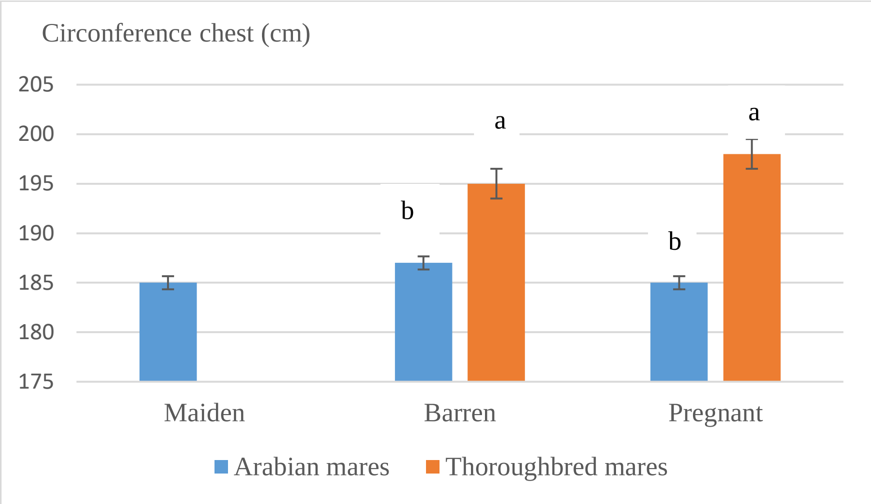


Figure 3. Variation of the mare’s chest circumference according to the age (Means±SD).



a,b : p<0.05

Figure 4. Variation of the mare's chest circumference according to the status (Means±SD).

The results showed that the pregnancy rate was higher in Thoroughbred mares compared to the Arabian ones (p<0.01).

CONCLUSIONS

According to these findings, the conformation parameters were improved in Thoroughbred mares and which explains their higher pregnancy rate compared to the Arabian mares. These differences could be due to the breed's genetic traits.

REFERENCES

AFDA, 2018. Equine reproduction. A guide for farmers and small breeders. 4th edition, June 2018. Farm management and rural development program, Teagasc, Mellows campus, Athenry, Co Galway.

Ravell D.G., 1999. Measuring and estimating the weight of horses with tapes, formulae and by visual assessment. Equine Vet. Educ., 11(6): 314-317.

Papa F.O., Melo C.M., Monteiro G.A., 2014. Equine perinal and vulvar conformation correction using a modification of Pouret's Technique. J. Equine Vet. Sci?, 34: 359-464.

Talluri T.R., Ravi S.k., Singh J., Legha R.A., Pal Y., 2015. Application of Caslick's Index in Predicting the Mare Fertility. Journal of Animal Research, 5(2): 219-222.

ENHANCING NUTRIENT COMPOSITION OF ROSEHIP PULP VIA ENZYMATIC SOLID-STATE FERMENTATION

Emrah GÜNGÖR,[1] Şevket ÖZLÜ[1], Bahar GÜNGÖR[2], Aydın ALTOP[1], Güray ERENER[1]

[1] Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Turkiye
[2] Ondokuz Mayıs University, Faculty of Science, Department of Molecular Biology and Genetics, Samsun, Turkiye

Corresponding author e-mail: emrah.gungor@omu.edu.tr

ABSTRACT

This study investigated the effects of exogenous enzymes on the nutrient composition of fermented rosehip pulp in the solid-state fermentation method. The research was conducted in a 3 x 2 factorial experiment. Three different fermentation periods were employed: 3, 5 and 7 days. Two different enzyme additions (yes, no) were made in each period. Five replicates were conducted for each treatment group, with the total number of samples reaching 35, including the control group. Before fermentation, the rosehip pulp was ground to a particle size of 1 mm and added to the fermenter. To facilitate microbial growth, 85.5 g of nutritional salt (glucose:urea:$(NH_4)_2SO_4$:peptone:KH_2PO_4:$MgSO_4.7H_2O$ = 4:2:6:1:4:1) was added to 1 lt of distilled water. The samples were sterilized at 121 °C for 15 minutes. The enzyme used in this study was obtained from a private company and comprises a combination of phytase, xylanase, and beta-glucanase. The enzyme was then added to a quantity of 1 g to each fermentation media and sterilized via UV light. *L. acidophilus* was incubated in a shaking incubator at 30 °C and 120 rpm for 48 hours using MRS broth. Then, 1 ml of the *L. acidophilus* culture (10^8 cfu/ml) was added to the sterilized fermenters. The highest crude protein, ash, and the lowest crude fiber were observed in the group fermented for seven days and enzyme was used ($P < 0.001$). The highest ether extract level was detected in the unfermented sample ($P < 0.001$). This research also corroborates these findings, as crude protein levels increased and crude fiber levels decreased. The optimal fermentation time for rosehip pulp with *L. acidophilus* was seven days, with the addition of enzymes.

Keywords: Solid-state fermentation, rosehip pulp, *L. acidophilus*, enzyme

INTRODUCTION

Rosehip is a shrub-shaped plant belonging to the *Rosaceae* family, which can grow in many regions of Turkey. The pulp is a waste product after juice extraction from the rosehip fruit. Rosehip contains high levels of vitamin C and vitamins A, B1, B2, B6, D, E, and K (Koczka et al., 2018). In addition, rose hips are a rich source of color substances, including lycopene, β-carotene, β-cryptoxanthin, gazaniaxanthin, and zeaxanthin (Grigorova et al., 2021).

Rosehip also contains potassium, phosphorus, calcium, magnesium, manganese, and zinc minerals (*Ercisli, 2007*). Rosehip seeds are notable for their high content of polyunsaturated fatty acids, including linoleic acid (45-55%), α-linoleic acid (18-32%), and oleic acid (13-20%) (Koczka et al., 2018). Additionally, rosehip is a rich source of phenolic compounds, including tannins, flavonoids (quercetin, catechin, chemical, rutin), phenolic acids (ellagic acid, protocatechuic acid, chlorogenic acid), and anthocyanins (Koczka et al., 2018). Rosehip pulp contains 10.8% crude protein, 4.6% ether extract, 2.4% ash, 55.8% crude fiber, and a total energy level of 4800 kcal (Vlaicu et al., 2018). Although rosehips are rich in beneficial compounds, their high fiber content limits their use, particularly in monogastric animals. The solid-state fermentation method has been demonstrated to be an effective means of eliminating anti-nutritional factors (Altop et al., 2019).

Fermentation applications utilizing microorganisms, including bacteria, fungi, and yeasts, can generally be divided into submerged and solid-state fermentation (Pandey, 2003). Solid-state fermentation is defined as the growth of microorganisms on moistened solid substrates without free water. This process is preferred to submerged fermentation due to its economic, abundant, and cheap substrates and its relatively lower risk of contamination (Sargın, 2003; *Pérez-Guerra et al., 2003). Solid-state fermentation is a process by which the nutritional composition of various feedstuffs can be enhanced, antinutritional factors reduced, phenolic substance content increased, enzyme activity augmented, and the in vitro digestibility of the feedstuffs improved (Güngör & Erener, 2023; Altop et al., 2018; Altop et al., 2021; Güngör et al., 2020).* This study investigated the effects of exogenous enzymes on the nutrient composition of fermented rosehip pulp in the solid-state fermentation method.

MATERIAL AND METHOD

The research was conducted in a 3 x 2 factorial experiment. Three different fermentation periods were employed: 3, 5 and 7 days. Two different enzyme additions (yes, no) were made in each period. Five replicates were conducted for each treatment group, with the total number of samples reaching 35, including the control group. The experimental plan of the research is presented in Table 1.

Table 1. Plan of the research

<table>
<tr><td colspan="2" rowspan="2"></td><td colspan="4">Days</td></tr>
<tr><td>0 days</td><td>3 Days</td><td>5 days</td><td>7 days</td></tr>
<tr><td rowspan="2">Enzyme</td><td>With enzyme</td><td></td><td>Fermented with enzyme for 3 days</td><td>Fermented with enzyme for 5 days</td><td>Fermented with enzyme for 7 days</td></tr>
<tr><td>Without enzyme</td><td>Control</td><td>Fermented without enzyme for 3 days</td><td>Fermented without enzyme for 5 days</td><td>Fermented without enzyme for 7 days</td></tr>
</table>

The rosehip pulp used in the study was obtained from a local business, and the samples were dried at 75 °C. The samples were stored at 4 °C until use in the study. The microbial inoculant, *L. acidophilus* (ATCC 4356), was obtained from the American Type Culture Collection (ATCC).

Before fermentation, the rosehip pulp was ground to a particle size of 1 mm and added to the fermenter. Subsequently, 100 g of the ground rosehip pulp and 500 ml of distilled water were added to each fermenter. To facilitate microbial growth, 85.5 g of nutritional salt (glucose:urea:(NH4)2SO4:peptone:KH2PO4:MgSO4.7H2O = 4:2:6:1:4:1) was added to 1 lt of distilled water (Cao et al., 2012). Subsequently, the samples were sterilized at 121 °C for 15 minutes. The enzyme used in this study was obtained from a private company and comprises a combination of phytase, xylanase, and beta-glucanase. The enzyme was then added to a quantity of 1 g to each fermentation media and sterilized via UV light.

L. acidophilus was incubated in a shaking incubator at 30 °C and 120 rpm for 48 hours using MRS broth (Güngör & Erener, 2020). Then, 1 ml of the *L. acidophilus* culture (10^8 cfu/ml) was added to the sterilized fermenters. The inoculated samples were fermented at 30°C for the specified duration (3–5–7 days). Once the fermentation process had been completed, dry matter, ash, crude protein, crude fiber, and ether extract analyses were conducted by Akyıldız (1984).

The data obtained from the research was subjected to analysis of variance using the GLM ANOVA/MANOVA procedure in the statistical software Statistica (1984) in a 3 x 2 factorial arrangement. This involved three fermentation periods (3, 5 and 7 days), two enzyme additions (yes or no), and a randomized-block design. Once the statistical significance of the differences between the groups had been established ($P < 0.05$), Tukey's HSD test was used to identify the specific group to which the difference applied ($P = 0.05$).

RESULTS AND DISCUSSION

The nutrient composition of rosehip pulp fermented using *L. acidophilus* in a solid-state fermentation method was found to be positively affected at the conclusion of the study. The highest crude protein, ash, and the lowest crude fiber were observed in the group fermented for seven days and enzyme was used ($P < 0.001$). The primary objective of fermentation studies in animal feeds is to reduce crude fiber levels and increase crude protein levels (Wu et al., 2015). The observed increase in crude protein content may be attributed to microbial protein production by microorganisms (Özlü and Altop, 2023). These findings align with those of Hajimohammadi et al. (2020), where sesame meal was fermented with *S. cerevisiae* and *L. acidophilus*, resulting in a notable increase in crude protein levels. Crude fiber components are structural carbohydrates found in the cell wall of plants and are present at high levels in agricultural wastes (Graminha et al., 2008). Crude fiber includes cellulose, hemicellulose, lignin, pectin, and xylan compounds (Özlü and Altop, 2023). The reduction of these compounds increases the digestibility of feeds. The reduction of crude fiber content in the solid-state fermentation method is possible thanks to the use of enzymes such as cellulase, hemicellulase, and β-glucosidase (Wu et al., 2015). This reduction is achieved both by the use of enzymes and by enzymes produced by microorganisms (Chuang et al., 2019; Maiorano et al., 2022).

The increase in ash level is comparable to that observed by Özlü and Altop (2023). This increase may be attributed to the relative increase in mineral content resulting from the consumption of organic material in the rosehip pulp by microorganisms and the nutritional salt added to the fermentation medium (Rajesh & Raj, 2010). The highest ether extract level was detected in the unfermented sample ($P < 0.001$). Similarly, Chi and Cho (2016) reported that the ether extract content of soybean meal fermented with L. acidophilus decreased.

CONCLUSION

It has been demonstrated that fermentation has a positive effect on the nutrient composition of various agricultural wastes, including rosehip pulp. This process allows for the increased use of feedstuffs in diets while also conferring additional benefits due to the probiotic effects of microorganisms. This research also corroborates these findings, as crude protein levels increased and crude fiber levels decreased. The optimal fermentation time for rosehip pulp with L. acidophilus was seven days, with the addition of enzymes.

REFERENCES

Akyıldız, A. (1984). *Yemler bilgisi laboratuvar kılavuzu.* Ankara: Ankara Üniversitesi Ziraat Fakültesi Yayınları.

Altop, A., Coskun, I., Filik, G., Kucukgul, A., Bekiroglu, Y. G., Cayan, H., & Gungor, E. (2018). Amino acid, mineral, condensed tannin, and other chemical contents of olive leaves (Olea europaea L.) processed via solid-state fermentation using selected Aspergillus niger strains.

Altop, A., Güngör, E., & Erener, G. (2021). Different Aspergillus niger Strains and Inoculum Levels Affect the Nutritional Composition of Olive Leaves in Solid-state Fermentation. *Turkish Journal of Agriculture-Food Science and Technology*, *9*(12), 2303-2307.

Altop, A., Güngör, E., and Erener, G., 2019. Improvement of nutritional quality of some oilseed meals through solid-state fermentation using Aspergillus niger, Turkish Journal of Agriculture-Food Science and Technology, 7, 1411-1414.

Chi CH, Cho SJ. Improvement of bioactivity of soybean meal by solid-state fermentation with *Bacillus amyloliquefaciens* versus *Lactobacillus* spp. and *Saccharomyces cerevisiae*. LWT-Food Science and Technology. 2016;68:619-25.

Chuang WY, Lin WC, Hsieh YC, Huang CM, Chang SC, Lee TT. Evaluation of the combined use of *Saccharomyces cerevisiae* and *Aspergillus oryzae* with phytase fermentation products on growth, inflammatory, and intestinal morphology in broilers. Animals. 2019;9(12):1051.

*Ercisli S. Chemical composition of fruits in some rose (*Rosa *spp.) species. Food Chemistry, 104, 4: 1379-1384, 2007.*

Graminha E, Gonçalves A, Pirota R, Balsalobre M, Da Silva R, Gomes E. Enzyme production by solid-state fermentation: Application to animal nutrition. Animal Feed Science and Technology. 2008;144(1-2):1-22.

Grigorova S, Gjorgovska N, Levkov V. Effects of rosehip feed supplementation on egg quality parameters, yolk lipid oxidation, and blood parameters of laying hens. Iranian Journal of Applied Animal Science, 11, 4: 827-833, 2021.

Güngör, E., & Erener, G., (2023). Katı Faz Fermantasyonunun Ceviz Küspesinin Besinsel Kompozisyonu ve İn vitro Sindirilebilirliği Üzerine Etkisi. *Türkiye Klinikleri,* 2023, 64-69.

Güngör, E., Altop, A., & Erener, G. (2020). Improvement in the nutritional composition and nutrient digestibility of pomegranate (Punica granatum L.) seed by Bacillus subtilis and Aspergillus niger solid-state fermentation. Anadolu Tarim Bilimleri Dergisi, 35(3), 268-273.

Hajimohammadi A, Mottaghitalab M, Hashemi M. Effects of microbial fermented sesame meal and enzyme supplementation on the intestinal morphology, microbiota, pH, tibia bone and blood parameters of broiler chicks. Italian Journal of Animal Science. 2020;19(1):457-67.

Koczka N, Stefanovits-Bányai É, Ombódi A. Total polyphenol content and antioxidant capacity of rosehips of some Rosa species. Medicines, 5, 3: 84, 2018.

Li Y, Guo B, Wu Z, Wang W, Li C, Liu G, Cai H. Effects of fermented soybean meal supplementation on the growth performance and cecal microbiota community of broiler chickens. Animals. 2020;10(6):1098.

Maiorano G, Ramires FA, Durante M, Palamà IE, Blando F, De Rinaldis G. The controlled semi-solid fermentation of seaweeds as a strategy for their stabilization and new food applications. Foods. 2022;11(18):2811.

Özlü, Ş., & Altop, A., (2023). Çeşitli Probiyotik Mikroorganizmalar Tarafından Fermente Edilmiş Zeytin Yapraklarının Besin Değeri ve In vitro Sindirilebilirliği. *Türkiye Klinikleri*, 2023, 70-77.

Pandey A. Solid-state fermentation. Biochemical Engineering Journal, 13, 2-3: 81-84, 2003.

Pérez-Guerra N, Torrado-Agrasar A, López-Macias C, Pastrana L. Main characteristics and applications of solid substrate fermentation. Electronic Journal of Environmental, Agricultural and Food Chemistry, 2, 3: 343-350, 2003.

Rajesh N, Raj RP. Value addition of vegetable wastes by solid-state fermentation using *Aspergillus niger* for use in aquafeed industry. Waste Management. 2010;30(11):2223-7.

Sargın S. Katı kültür fermantasyonu ile ksilanaz enzim üretiminin optimum koşullarının belirlenmesi. Fen Bilimleri Enstitüsü Biyoteknoloji Anabilim Dalı, 2003.

Vlaicu PA, Panaite TD, Voicu I, Turcu RP, Olteanu M, Ropota M. Determining the feeding value of some food industry by-products. Scientific Papers: Animal Science & Biotechnologies/Lucrari Stiintifice: Zootehnie si Biotehnologii, 51, 1, 2018.

Wu Q, Wang Z, Wang G, Li Y, Qi Y. Effects of feed supplemented with fermented pine needles (*Pinus ponderosa*) on growth performance and antioxidant status in broilers. Poultry Science. 2015;94(6):1138-44.

IMPACT OF ENZYMATIC TREATMENT ON SOYBEAN MEAL FERMENTATION ON NUTRITIONAL COMPOSITION

Bahar GÜNGÖR[2], Şevket ÖZLÜ[1], Emrah GÜNGÖR[1], Aydın ALTOP[1], Güray ERENER[1]

[1] *Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Turkiye*

[2] *Ondokuz Mayıs University, Faculty of Science, Department of Molecular Biology and Genetics, Samsun, Turkiye*

Corresponding author e-mail: bahargaripoglugungor@gmail.com

ABSTRACT

This study investigated the effects of exogenous enzymes on the nutrient composition of fermented soybean meal in the solid-state fermentation method. The research was conducted in a 3 x 2 factorial experiment, with three different fermentation periods (3, 5 and 7 days), two different enzyme additions (yes, no) in each period, and five replicates in each group for a total of 35 samples, including the control group. Before fermentation, the soybean meal was ground to a particle size of 1 mm and added to the fermenter. Subsequently, 100 g of the ground soybean meal and 500 ml of distilled water were added to each fermenter. The enzyme used in this study comprises a combination of phytase, xylanase, and beta-glucanase. The enzyme was then added to a quantity of 1 g to each fermentation media and sterilized via UV light. 1 ml of the *L. acidophilus* culture (10^8 cfu/ml) was added to the sterilized fermenters. The inoculated samples were fermented at 30 °C for the specified duration (3–5–7 days). The study concluded that the nutrient composition of soybean meal was positively affected. Overall, crude protein and ether extract levels increased significantly ($P<0.001$). Additionally, a fermentation time x enzyme interaction was observed regarding nutrient composition ($P<0.001$). The highest crude protein level was in the sample fermented for three days without an enzyme ($P<0.001$). The lowest crude fiber level was in the fermented for three or five days without an enzyme ($P<0.001$). The highest ether extract level was in the seven days with enzyme, however same sample had lowest crude protein level ($P<0.001$). The optimal fermentation period for soybean meal with *L. acidophilus* and without enzymes was found to be three days.

Keywords: Solid-state fermentation, soybean meal, *L. acidophilus*, enzyme

INTRODUCTION

Meat and meat products are crucial protein sources for ensuring optimal and balanced nutrition for the global population. With the anticipated 58% surge in demand for meat products due to population growth (Makkar et al., 2014), the production of broiler chickens in Turkey plays a vital role in meeting this escalating need. To keep pace with the rising demand, there is a necessity to elevate the production rate of broiler chickens, which in turn will escalate the requirement for feedstuffs. As some feed ingredients used in animal nutrition also have applications in human nutrition, it is imperative to explore alternative sources.

In poultry farming, feed expenses constitute around 60-70% of the total costs. By incorporating alternative materials and feed supplements in lieu of conventional feed ingredients, it's plausible to curtail feed expenditure. The practice of using antibiotics as growth promoters in broiler feeds can pose risks and leave residues in the end product. Consequently, novel alternative additives have been developed post-2006 to amplify broiler performance, safeguard intestinal health, and provide supplementary benefits. Notably, the utilization of diverse raw materials categorized as agricultural waste has garnered attention in recent years.

Soybean (*Glycine max*) is a plant that is cultivated in numerous regions across the globe and is utilized in the oil industry (Smith & Huyser, 1987). Once the oil has been extracted, the remaining portion is referred to as soybean meal and is utilized in animal nutrition. SM is one of the most highly regarded vegetable protein sources utilized in animal nutrition (Banaszkiewicz, 2011). Consequently, it is a crucial ingredient in livestock diets. Soybean meal is utilized in animal nutrition at a rate of 20-25% in laying hens, 25-35% in broiler chickens, 10-20% in pigs, and 15-20% in cattle (Brzóska, 2009; Brzóska et al., 2009). Soybean meal is obtained through a variety of processes (Berk, 1992). The differences in the extraction methods result in variations in the nutrient composition of the meal (Banaszkiewicz, 2011). In general, it has a crude protein content of 44-56%, an ether extract content of 0-3%, and a crude fiber content of 4-8% (Ensminger et al., 1990; NRC, 1998; Poultry Feeding Standards, 2005). The high protein content and high amino acid digestibility by monogastrics indicate that SM is a high-quality protein source (Ravindran et al., 2005). Nevertheless, the presence of anti-nutritional factors in its structure represents a limitation to its use in livestock nutrition (Liener, 1994; Liener, 2000; Hoeck et al., 2000; Fasina et al., 2003). While numerous feed processing methods exist to eliminate these factors, the fermentation method has proven to be particularly effective.

Solid-state fermentation (SSF) is defined as the growth and metabolic activity of microorganisms on solid substrates that have been moistened without free water (Mitchell et al., 2000). The SSF process facilitates the conversion of nutrients through the enzymatic interactions of microorganisms on waste material, resulting in an enhancement of the nutrient composition of the plant material. It reduces the presence of anti-nutritional compounds and increases their bioavailability (Steudler et al., 2019). This process can enhance the digestibility of waste material (Özlü & Altop, 2023) and the bioavailability of nutrients (Güngör & Erener, 2023). In studies on fermentation, bacteria, fungi, or yeasts are typically preferred as inoculants, with the combination of two or more types of microorganisms being the preferred inoculant (Li et al., 2020; Saleh et al., 2021). This study investigated the effects of exogenous enzymes on the nutrient composition of fermented soybean meal in the solid-state fermentation method.

MATERIAL AND METHOD

The study was carried out in a 3 x 2 factorial design involving three fermentation periods (3, 5 and 7 days), two enzyme additions (yes or no) per period, and five replicates per group totaling 35 samples, which includes the control. The experimental layout is detailed in Table 1.

The soybean meal utilized was sourced from a local company, dried at 75 °C, and stored at 4 °C until needed. The microbial inoculant, *L. acidophilus* (ATCC 4356), was procured from the American Type Culture Collection (ATCC).

Prior to fermentation, the soybean meal was ground to 1 mm particle size and placed in the fermenter. Subsequently, each fermenter received 100 g of ground soybean meal and 500 ml of distilled water. To promote microorganism growth, a mixture of nutritional salt (glucose:urea:(NH4)2SO4:peptone:KH2PO4:MgSO4.7H2O = 4:2:6:1:4:1) was added. After sterilization at 121 °C for 15 minutes, 1 g of a composite enzyme (phytase, xylanase, and beta-glucanase) was included in each fermentation medium and sterilized with UV light.

Table 1. Plan of the research

		Days			
		0 days	3 Days	5 days	7 days
Enzyme	With enzyme		Fermented with enzyme for 3 days	Fermented with enzyme for 5 days	Fermented with enzyme for 7 days
	Without enzyme	Control	Fermented without enzyme for 3 days	Fermented without enzyme for 5 days	Fermented without enzyme for 7 days

L. acidophilus was cultivated in a shaking incubator at 30 °C and 120 rpm for 48 hours in MRS broth. Subsequently, 1 ml of the *L. acidophilus* culture (10^8 cfu/ml) was introduced into the sterilized fermenters. The fermenting samples were left at 30°C for the designated duration (3–5–7 days). Following fermentation completion, analyses for dry matter, ash, crude protein, crude fiber, and ether extract were performed based on Akyıldız's method (1984).

The data collected underwent analysis of variance using the GLM ANOVA/MANOVA procedure with a 3 x 2 factorial design (three fermentation periods, two enzyme additions), in Statistica (1984). Statistical significance ($P < 0.05$) indicated variations between groups, which were further examined using Tukey's HSD test at a significance level of $P = 0.05$ to pinpoint the differing groups.

RESULT AND DISCUSSION

The study concluded that the nutrient composition of soybean meal was positively influenced. Results were comparable to Altop et al. (2019). Crude protein level significantly increased (P<0.001). A fermentation time x enzyme interaction affected nutrient composition (P<0.001). Crude fiber, an anti-nutritional factor for monogastric animals, decreased (P<0.001). The highest crude protein level was in the sample fermented for three days without an enzyme (P<0.001). Feedstuffs' nutrient composition is positively impacted by solid-state fermentation (Wang et al., 2017; Altop et al., 2018). Li et al. (2020) found increased crude protein levels in soybean meal fermented with *L. acidophilus*, consistent with prior results due to microbial protein production.

The lowest crude fiber level was in the fermented for three or five days without an enzyme (P<0.001). The second lowest was in the sample five days without enzyme (P<0.001). Güngör et al. (2017) reported cherry kernels' crude fiber levels varied when fermented with different microorganisms. A relative increase in crude fiber rates may be due to microorganism-substrate incompatibility or decreased nutrients (carbohydrates). The highest ether extract level was in the seven days with enzyme, however same sample had lowest crude protein level (P<0.001).

CONCLUSION

Fermentation has been shown to improve the nutrient content of different agricultural wastes, such as soybean meal. This enhances the utilization of feed in diets and provides additional benefits through the probiotic effects of microorganisms. Recent research confirms these findings, showing an increase in crude protein levels, stable ether extract levels, and a decrease in crude fiber levels. The optimal fermentation period for soybean meal with *L. acidophilus* and without enzymes was found to be three days.

REFERENCES

Akyıldız, A. (1984). *Yemler bilgisi laboratuvar kılavuzu.* Ankara: Ankara Üniversitesi Ziraat Fakültesi Yayınları.

Altop A, Güngör E, Erener G. Aspergillus niger may improve nutritional quality of grape seed and its usability in animal nutrition through solid-state fermentation. International Advanced Researches and Engineering Journal. 2018;2(3):273-277.

Altop, A., Güngör, E., and Erener, G., 2019. Improvement of nutritional quality of some oilseed meals through solid-state fermentation using Aspergillus niger, Turkish Journal of Agriculture-Food Science and Technology, 7, 1411-1414

Banaszkiewicz, T. (2011). Nutritional value of soybean meal. *Soybean and nutrition, 12*, 1-20.

BrzóskaF. .2009Is it possible to replace GMO protein with other protein source, Part I (in Polish). Wiadomości Zootechniczne, Vol. XLVII, 1391731-8068.

BrzóskaF.KoreleskiJ.KorolW.2009Possible consequences of a ban on genetically modified (GM) soybean in animal feed (in Polish). Wiadomości Zootechniczne, Vol. XLVII, 33101731-8068

EnsmingerM. E.OldfieldJ. E..HeinemannW. W.1990Feeds and Nutrition. The Ensminger Publishing Company, 0-94121-808-2California

FasinaY. O.ClassenH. L.GarlichJ. D.SwaisgoodH. E.ClareD. A.2003Investigating the possibility of monitoring lectin levels in commercial soybean meals intended for poultry feeding using steam-heated soybean meal as a model. Poultry Science, 826486560032-6791

Güngör, E., Altop, A., Öztürk, E., and Erener, G., 2017. Nutritional changes of sour cherry (Prunus cerasus) kernel subjected to Aspergillus niger solid-state fermentation, Tekirdağ Ziraat Fakültesi Dergisi.

Güngör E, Erener G. Katı faz fermantasyonunun ceviz küspesinin besinsel kompozisyonu ve in vitro sindirilebilirliği üzerine etkisi. Balevi T, editör. Kanatlıların Beslenmelerinde Son Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2023. p.64-9.

HoeckJ. A.FehrW. R.MurphyP. A.WelkeG. A.2000Influence of genotype and environment on isoflavone contents of soybean. Crop Science, 4048510001-1183X

Li Y, Guo B, Wu Z, Wang W, Li C, Liu G, Cai H. Effects of fermented soybean meal supplementation on the growth performance and cecal microbiota community of broiler chickens. Animals. 2020;10(6):1098.

LienerI.E.1994Implications of anti-nutritional components in soybean foods. Critical Reviews in Food Science and Nutrition, 3431671040-8398

LienerI.E.2000Non-Nutritive Factors and Bioactive Compounds in Soy. Soy in Animal nutrition Symposium (August 45Chicago, IL), (Ed. J.K. Drackley), Fed. An. Sci. Societies, Savoy, 101884706010USA.

Makkar, H. P., Tran, G., Heuzé, V. ve Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, 197, 1-33.

Mitchell, D. A., Krieger, N., Stuart, D. M., & Pandey, A. (2000). New developments in solid-state fermentation: II. Rational approaches to the design, operation and scale-up of bioreactors. *Process Biochemistry*, *35*(10), 1211-1225.

NationalResearch.CouncilN. R. C.1998Nutrient Requirements of Swine(Tenth revised Ed). National Academy Press, 0-30905-993-3DC. USA

Özlü Ş, Altop A. Çeşitli probiyotik mikroorganizmalar tarafından fermente edilmiş zeytin yapraklarının besin değeri ve in vitro sindirilebilirliği. Balevi T, editör. Kanatlıların Beslenmelerinde Son Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2023. p.70-4.

Poultry Feeding Standards.2005Ed. The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, 8-39170-977-9onna /Warszawa, Poland

Saleh, A. A., Shukry, M., Farrag, F., Soliman, M. M. ve Abdel-Moneim, A. M. E. (2021). Effect of feeding wet feed or wet feed fermented by *Bacillus licheniformis* on growth performance, histopathology and growth and lipid metabolism marker genes in broiler chickens. *Animals*, 11 (1). 83.

SmithK.J.HuyserW.1987World distribution and significance of soybean. In: Soybeans: Improvement, Production, and Uses. Wilcox J.R. (ed), Second edition. Agronomy Monographs 16American Society of Agronomy, 1220-89118-090-7WI

Wang JH, Guo H, Zhang TR, Wang H, Liu BN, Xiao S. Growth performance and digestion improvement of juvenile sea cucumber Apostichopus japonicus fed by solid-state fermentation diet. Aquaculture Nutrition. 2017;23(6):1312-1318.

EFFECTS OF SOME ENVIRONMENTAL FACTORS ON GROWTH TRAITS OF BUFFALO CALVES

İbrahim Cihangir OKUYUCU[1], Hüseyin ERDEM[1]

[1]Department of Animal Science, Faculty of Agriculture, Ondokuz Mayis University, 55139, Samsun, Türkiye

Corresponding author e-mail: cihangir.okuyucu@omu.edu.tr

ABSTRACT

The aim of this study was (i) to evaluate the growth traits of buffalo calves [Live weight (LW), Chest girth (CG) Chest depth (CD), Chest width (CW), Withers height (WH), Rump height (RH) and Body length (BL)] in the 0-6 mo period and (ii) to investigate the effects of some environmental factors such as, body condition score (BCS), calving season, gender and parity on these traits. The study was conducted in 5 farms in the Samsun province, located in the Black Sea region in Türkiye. A total of 86 Anatolian buffalo cows and calves were selected. The body condition of the cows was scored subjectively at 15–20 d before calving. Based on the statements of the owner farmers, parity, calving seasons, gender and calf birth dates were recorded. Also, LW, CG, CD, CW, WH, RH and BL of calves were measured once a month for 6 mo. Data of evaluated variables were analysed using a general linear model procedure and t-test. The LW of the calves positively correlated with other growth traits. LW values of male calves were found to be higher than female calves at birth and 30 d. LW and BL values calves of cows with high BCS were higher than calves of those with low BCS at all growth periods. Except from birth, RH values calves of cows with high BCS were higher than calves of those with low BCS at other growth periods. Similarly, other growth traits were affected by BCS in different growth periods. Calving season affected the LW and CD values of calves at birth. At birth, the LW values of the calves of cows with parity of ≥5 were higher than the calves of cows with parity of 1. At the same period, the CG and WH values of the calves of cows with parity of 4 and ≥5 were higher than the calves of cows with parity of 1. It was concluded that parity, calving season, gender and BCS of the cow may affect the growth characteristics of calves at different growth periods.

Keywords: Anatolian buffaloes, Environmental factors, Calves, Growth traits

INTRODUCTION

As in dairy cattle farms, the most important part of sustainable production in buffalo farms is successful calf management (Okuyucu and Erdem, 2018; Kharkar et al., 2019). Cows that are culled from the herd due to various reasons such as old age, low productivity and disease are replaced by young buffalo heifers and production continues with these animals. Therefore, raising healthy buffalo calves with high growth performance is directly related to profitable and sustainable production.

To evaluate the growth performance of calves, buffalo breeders and researchers focus on live weight (LW), which is one of the most important indicators of growth, because birth weight and LW are related to survival as well as adaptation (Alkoyak and Öz, 2022). Although this trait is considered as the first measure of growth performance, chest girth (CG), chest depth (CD), chest width (CW), withers height (WH), rump height (RH) and body length (BL) are other body traits used in determining growth performance. These body traits are used as indicator traits or selection criteria in breeding selection programs. Moreover, these traits allow the animals that do not reach the targeted average body measurements in certain growth periods to be identified and culling from the herd (Şekerden, 2010).

It has long been known that the growth traits of buffalos are affected by both genetic factors and non-genetic environmental factors. Environmental factors may adversely affect the actual growth potential of the animal, thereby reducing the accuracy of the selection program applied (Alkoyak and Öz, 2022). Therefore, environmental factors affecting yield traits should be carefully examined. Previous studies have emphasized that many environmental factors such as gender, parity, calving season and calving year have an effect on the growth traits of calves (Şekerden and Tapkı, 2003; Gupta et al., 2012; Hossein-Zadeh et al., 2012; Alkoyak and Öz, 2022). In a study conducted on Egyptian buffalo calves, El-Bramony et al. (2008) reported that the birth weight (BW) of female calves was lower than that of male calves. In the same study, it was determined that as the parity of the cow increased, the BW of the calf also increased. In the studies conducted on Anatolian buffalo calves (Erdem et al., 2015; Alkoyak and Öz, 2022), it was noted that the calving season had a significant effect on BW. Recent studies have focused on environmental factors such as parity, calving season and gender affecting the growth traits of buffalo calves (Şekerden and Tapkı, 2003; Gupta et al., 2012; Hossein-Zadeh et al., 2012; Alkoyak and Öz, 2022). To our knowledge, there is no information on the effects of dry period BCS of the cow on the growth traits (LW, CG, CD, CW, WH, RH and BL) of calves in addition to these environmental factors. Our hypothesis was that environmental factors such as gender, cow’s BCS, calving season and parity had an effect on the growth characteristics of calves in the 0-6 growth period.

The aim of this study was (i) to determine the correlations between LW and some growth traits (CG, CD, CW, WH, RH and BL), (ii) to evaluate the growth traits of buffalo calves in the 0-6 mo period and (iii) to investigate the effects of some environmental factors such as, body condition score (BCS), calving season, gender and parity on these traits.

MATERIAL AND METHOD

All the experimental procedures were performed according to the guidelines and approved by the local Ethics Committee of Ondokuz Mayis University (Permit No: 2018/21).

This study was conducted in five commercial dairy buffalo farms in the Bafra district of Samsun, where buffalo herds are densely populated,Türkiye. These farms were selected within a 15 km radius of Bafra district to facilitate data collection and body trait measurement. A total of 86 Anatolian buffalo cows and female (n=35) and male (n=51) calves were used. Buffalo cows on the farms were (i) housed in semi open free stall barns and (ii) fed a total mixed ration consisting of meadow hay, maize silage, paddy straw and commercial concentrate. The buffalo cows were fed with the same feeds both during the dry period and the lactation period.

Body condition status of pregnant buffaloes was scored on a scale of 1 to 5 during the 15–20 d before birth (Singh et al., 2017). In this range, 5 scales were defined: Emaciated/poor: 1-1.5; Thin/moderate: 2.0-2.5; Average/good: 3.0-3.5; Fat: 4.0-4.5; Obese/ grossly fat: 5.0. After birth, each calf was housed with its dam for five days. Then, the calves were separated from their dams in the following days. The buffaloes were fed with the same feeds both during the dry period and the lactation period. The calves were group-housed (6 calves in each pen) for 6 mo. During the research, calf's birth dates, calf's gender, dam's parity and calving season were recorded.

Buffalo cows that completed the transition from colostrum to normal milk were milked once per day in the morning. During milking, the teat cups of the milking machine were attached to two teats of the cow, while the other two teats were reserved for feeding the calf. After milking, the calves were kept with their dams for feeding. From 2 mo to 6 mo after calving, three teats of each cow were milked, while the other teat was reserved for feeding the calf. The calves in this study were weaned at 6 mo. From 10 days, calves were fed meadow hay and concentrate ad libitum.

To determine the growth traits of newborns, farms were visited within 24 h after birth. Then, the LW of the calves were measured using a digital scale. The CG was determined with a tape measure and CD, CW, WH, RH and BL was measured with a measuring stick. The investigated growth traits were measured at birth, 30 d, 60 d, 90 d, 120 d, 150 d and 180 d.

To reveal the relationships between factors and variables in the current data, the calving season was examined in 4 groups (Autumn, Winter Spring and Summer). Also, the parity was classified as 1, 2, 3, 4, and 5≤. The cow's BCS was calculated and those below the mean were defined as the first group (Low: <2.27) and those above the mean were defined as the second group (High: >2.27). Data were analysed using SPSS. The normality and homogeneity of the data obtained on the body traits of the calves were checked using the Kolmogorov-Smirnov and Levene's tests ($p > 0.05$).

The effects of gender, calving season and parity on body traits (LW, CG, CD, CW, WH, RH and BL) at birth, 30 d, 60 d, 90 d, 120 d, 150 d and 180 d were investigated using the model below.

$$Y_{ijkl}: \mu + a_i + b_j + c_k + e_{ijkl}$$

Where Y_{ijkl} = an observed value of LW, CG, CD, CW, WH, RH and BL; μ = the overall mean; a_i= the effect of the gender of the calves (i = Female, Male); b_j = the effect of calving season (j = Autumn, Winter, Spring, Summer); c_k = the effect of the parity (k = 1, 2, 3, 4 and 5≤); and e_{ijkl}= the random error.

To determine the effects of BCS of the cow and gender on body traits (LW, CG, CD, CW, WH, RH and BL) at birth, 30 d, 60 d, 90 d, 120 d, 150 d and 180 d were analyzed using the t-test.

RESULTS AND DISCUSSION

In the present study, LW value of buffalo calves at birth (Figure 1a) was lower than several previously reported studies (Murrah buffalo calves, Barbosa et al., 2006; Egyptian buffalo calves, El-Bramony et al., 2008), but higher than others (Surti calves, Sorathiya et al., 2009; Anatolian buffalo calves, Erdem et al., 2022; Alkoyak and Öz, 2022). In the present study, the CG, CW, WH and RH values at birth (Figure 1) were consistent with the values reported by Erdem et al. (2022), but the CD and BL values were not consistent.

The LW value at 30 days of age was consistent with the results of the study conducted by Erdem et al. (2022), but was not consistent with the other body characteristics examined. Also, LW value of buffalo calves at 180 d was higher than several previously reported studies (Surti calves, Sorathiya et al., 2009; Murrah calves, Gupta et al., 2012). However, the LW value of Anatolian buffalo calves at 180 days of age was lower than that reported in another previous study (Çelikeloğlu et al., 2015).

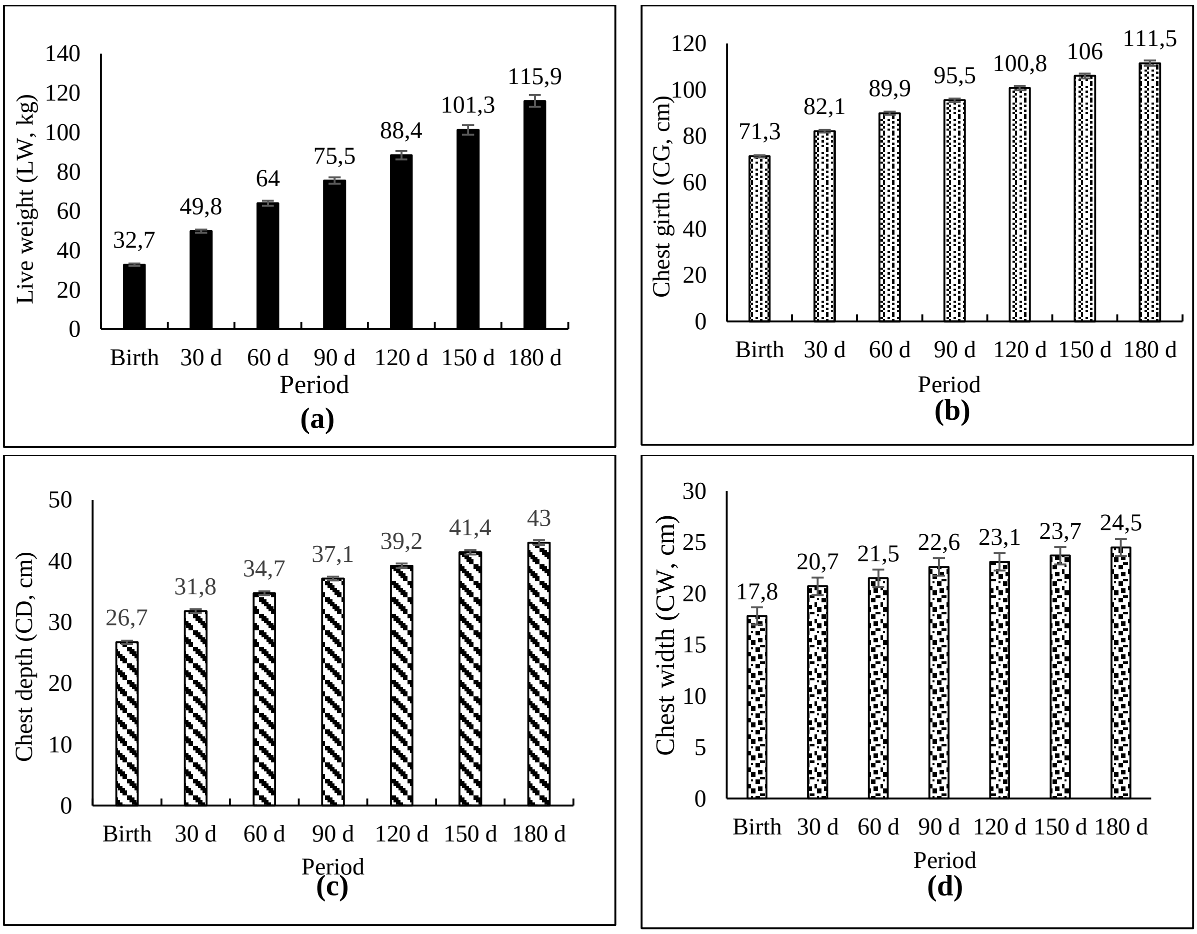


Figure 1. Mean values of live weight and body measurements of calves

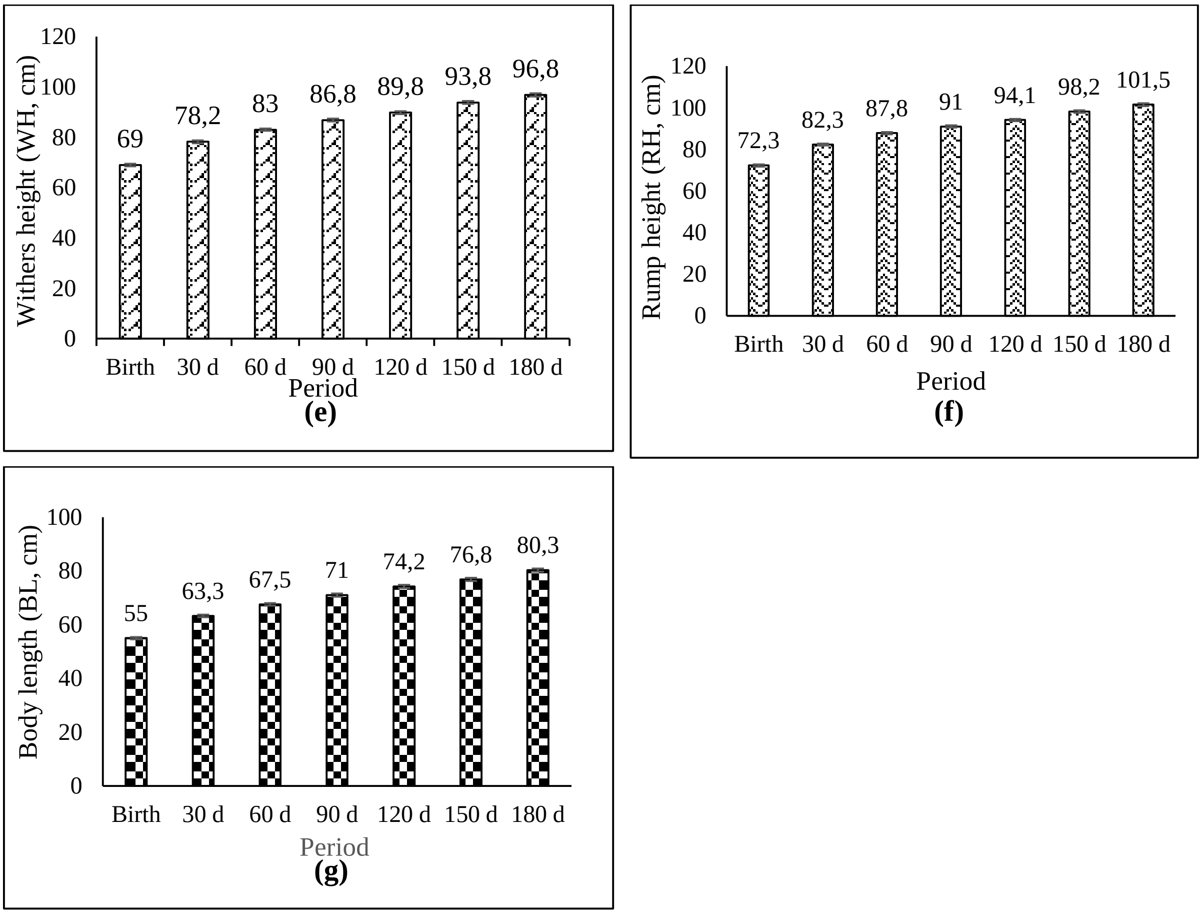


Figure 1. Cont.

In the present study, statistically significant positive correlation coefficients were determined between LW and other growth traits (Table 1).

Table 1. Correlation coefficients between live weight and some body traits

Periods	Body Traits					
	CG	CD	CW	WH	RH	BL
Birth	0.871 (p = 0.01)	0.478 (p = 0.01)	0.545 (p = 0.01)	0.707 (p = 0.01)	0.714 (p = 0.01)	0.639 (p = 0.01)
30 d	0.832 (p = 0.01)	0.472 (p = 0.01)	0.541 (p = 0.01)	0.684 (p = 0.01)	0.668 (p = 0.01)	0.499 (p = 0.01)
60 d	0.927 (p = 0.01)	0.787 (p = 0.01)	0.594 (p = 0.01)	0.735 (p = 0.01)	0.778 (p = 0.01)	0.599 (p = 0.01)
90 d	0.891 (p = 0.01)	0.793 (p = 0.01)	0.509 (p = 0.01)	0.779 (p = 0.01)	0.827 (p = 0.01)	0.684 (p = 0.01)
120 d	0.910 (p = 0.01)	0.820 (p = 0.01)	0.432 (p = 0.01)	0.760 (p = 0.01)	0.789 (p = 0.01)	0.766 (p = 0.01)
150 d	0.929 (p = 0.01)	0.802 (p = 0.01)	0.460 (p = 0.01)	0.835 (p = 0.01)	0.859 (p = 0.01)	0.699 (p = 0.01)
180 d	0.933 (p = 0.01)	0.769 (p = 0.01)	0.617 (p = 0.01)	0.829 (p = 0.01)	0.868 (p = 0.01)	0.801 (p = 0.01)

The effect of gender on LW values of buffalo calves at birth ($p = 0.048$) and 30 d of age ($p = 0.019$) was found to be significant, but this effect disappeared in the subsequent 60 d, 90 d, 120 d, 150 d and 180 d of growth periods (Table 2). During these growth periods, LW values of female calves were lower than males. The findings of the present study were consistent with previous studies on buffalo calves (Şekerden, 2010; Kul et al., 2018; Alkoyak and Öz, 2022). However, the effect of gender on other growth characteristics was statistically insignificant in all growth periods. The variation in LW values of female and male calves may have been caused by differences in physiological and/or endocrine systems (Alkoyak and Öz, 2022).

Table 2. Mean growth traits in different age periods according to gender

Period	Gender	LW	CG	CD	CW	WH	RH	BL
Birth	Female	31.1	70.2	26.3	17.4	68.3	71.4	55.0
	Male	33.8	72.0	26.9	18.1	69.5	72.6	55.4
	SEM	0.66	0.47	0.25	0.21	0.50	0.53	0.42
	p-value	p = 0.048	p = 0.064	p = 0.221	p = 0.120	p = 0.251	p = 0.429	p = 0.309
30 d	Female	47.5	81.6	31.6	20.3	77.5	81.6	62.2
	Male	52.0	82.5	31.9	20.9	78.6	82.9	64.1
	SEM	0.86	0.54	0.27	0.18	0.53	0.49	0.49
	p-value	p = 0.019	p = 0.390	p = 0.599	p = 0.130	p = 0.338	p = 0.211	p = 0.053
60 d	Female	61.6	89.3	34.7	21.2	82.8	86.5	67.0
	Male	65.7	90.3	34.7	21.8	83.1	86.9	67.9
	SEM	1.28	0.68	0.30	0.20	0.48	0.50	0.47
	p-value	p = 0.113	p = 0.470	p = 0.963	p = 0.123	p = 0.782	p = 0.679	p = 0.346
90 d	Female	73.5	95.3	37.1	22.3	86.5	90.7	70.7
	Male	76.8	95.6	37.1	22.7	87.1	91.2	71.3
	SEM	1.64	0.81	0.31	0.22	0.57	0.59	0.55
	p-value	p = 0.350	p = 0.857	p = 0.973	p = 0.382	p = 0.643	p = 0.669	p = 0.589
120 d	Female	87.2	101.1	39.3	22.7	89.6	94.2	74.0
	Male	89.2	100.7	39.1	23.4	90.0	94.0	74.1
	SEM	2.13	0.90	0.37	0.27	0.55	0.56	0.60
	p-value	p = 0.658	p = 0.839	p = 0.781	p = 0.226	p = 0.733	p = 0.903	p = 0.917
150 d	Female	100.3	106.3	41.4	23.2	93.2	97.7	76.8
	Male	101.9	105.8	41.4	23.9	94.2	98.5	76.9
	SEM	2.43	1.00	0.36	0.22	0.60	0.58	0.60
	p-value	p = 0.748	p = 0.796	p = 0.976	p = 0.122	p = 0.388	p = 0.519	p = 0.944
180 d	Female	115.6	111.9	43.3	23.8	97.2	102.0	80.2
	Male	116.1	111.3	42.8	24.9	96.5	101.1	80.4
	SEM	3.01	1.15	0.41	0.27	0.62	0.65	0.59
	p-value	p = 0.930	p = 0.769	p = 0.515	p = 0.056	p = 0.596	p = 0.502	p = 0.893

LW: Live weight; CG: Chest girth; CD: Chest depth; CW: Chest width; WH: Withers height; RH: Rump height;
BL: Body length

BCS is one of the important welfare assessment criteria that subjectively indicates the body fat reserves and nutritional status of the animal in all ruminants (Akdag et al., 2024). Therefore, BCS values in both lactation and dry period can affect milk yield, milk components (Akdag et al., 2024), fertility traits (Bezdíček et al., 2020), as well as the growth performance of calves. The mean BCS of cows (2.27) at the dry period was relatively low in our study. This shows that buffalo cows in the dry period were not fed adequately. Additionally, LW and other body traits of calves in some growth periods were affected by maternal BCS value in the dry period (Table 3). Calves of cows with high BCS had higher LW, WH and BL values at birth compared to those of cows with low BCS ($p = 0.027$). Similarly, calves of cows with high BCS had higher LW, WH and BL values at other growth periods compared to those of cows with low BCS. Except for the 180-d age period, calves of cows with high BCS had higher CG values compared to those of cows with low BCS in all other growth periods. Similarly, except for the 180-d age period, calves of cows with high BCS had higher WH values compared to those of cows with low BCS in all other growth periods. Also, except for the birth, calves of cows with high BCS had higher RH values compared to those of cows with low BCS in all other growth periods. Calves of cows with high BCS had higher BL values compared to those of cows with low BCS in all growth periods (Table 3). In a similar study conducted on Holstein cows and calves (Metin Kıyıcı, and Sarıaslan, 2023), as the BCS value of the cow increased, the BW and BL values of the calves also increased. The results of the present study were consistent with the findings reported by Metin Kıyıcı and Sarıaslan (2023). These results indicate that buffalo cows with good BCS values during the dry period have positive effects on the growth characteristics of the calves born. Therefore, it is thought that careful monitoring of the feeding and management practices and body condition of buffalos in the dry period can offer significant contributions to the performance of their calves during the growth period.

Table 3. Mean body traits of calves in different age periods according to body condition score of dam

Period	Groups	Body Traits							
		n	LW	CG	CD	CW	WH	RH	BL
Birth	Low	59	31.7	70.6	26.5	17.6	68.23	71.6	54.2
	High	27	34.9	72.7	27.2	18.3	70.7	73.7	56.8
	SEM		0.66	0.47	0.25	0.21	0.50	0.53	0.42
	p-value		p = 0.027	p = 0.033	p = 0.214	p = 0.104	p = 0.020	p = 0.066	p = 0.004
30 d	Low	58	48.3	81.2	31.5	20.6	77.0	81.3	62.5
	High	27	53.4	84.0	32.4	21.0	80.7	84.5	65.0
	SEM		0.86	0.54	0.27	0.18	0.53	0.49	0.49
	p-value		p = 0.005	p = 0.015	p = 0.105	p = 0.305	p = 0.001	p = 0.002	p = 0.015
60 d	Low	57	61.1	88.2	34.2	21.2	81.7	85.3	66.4
	High	27	70.2	93.3	35.7	22.2	85.8	90.0	69.9
	SEM		1.28	0.68	0.30	0.20	0.48	0.50	0.47
	p-value		p = 0.001	p < 0.001	p = 0.016	p = 0.017	p < 0.001	p < 0.001	p <0.001
90 d	Low	56	72.2	94.1	36.6	22.3	85.4	89.3	70.3
	High	26	82.7	98.4	38.2	23.3	90.0	94.5	72.6
	SEM		1.64	0.81	0.31	0.22	0.57	0.59	0.55
	p-value		p = 0.002	p = 0.013	p = 0.017	p = 0.033	p < 0.001	p < 0.001	p = 0.046
120 d	Low	55	83.3	99.1	38.6	22.7	88.9	92.8	73.0
	High	26	99.1	104.5	40.5	23.9	91.7	97.0	76.8
	SEM		2.13	0.90	0.37	0.27	0.55	0.56	0.60
	p-value		p = 0.002	p = 0.004	p = 0.017	p = 0.064	p = 0.014	p < 0.001	p = 0.002
150 d	Low	55	96.4	104.4	40.9	23.4	92.7	96.8	75.6
	High	26	111.6	109.4	42.5	24.2	96.2	101.2	79.4
	SEM		2.43	1.00	0.36	0.22	0.60	0.58	0.60
	p-value		p = 0.002	p = 0.017	p = 0.038	p = 0.120	p = 0.005	p < 0.001	p = 0.003
180 d	Low	55	111.1	110.2	42.7	24.4	95.7	100.2	79.5
	High	26	126.0	114.4	43.7	24.7	99.2	104.1	82.0
	SEM		3.01	1.15	0.41	0.27	0.62	0.65	0.59
	p-value		p = 0.020	p = 0.082	p = 0.251	p = 0.573	p = 0.573	p = 0.006	p = 0.004

LW: Live weight; CG: Chest girth; CD: Chest depth; CW: Chest width; WH: Withers height; RH: Rump height; BL: Body length

Another environmental factor examined, the calving season, statistically affected the LW values of calves at birth ($p = 0.048$; Table 4). The LW values of calves born in winter were highest at birth. However, it was determined that the LW value was highest in calves born in the summer months at 60 days of age ($p = 0.018$).

The CD values were higher in calves born in summer than in those born in autumn and winter at birth ($p = 0.028$), 30 d and 60 d ($p < 0.001$). Also, the CD values were higher in calves born in summer (43.4 cm) than in those born in spring (40.2 cm) at 150 d ($p = 0.015$). The CW values were higher in calves born in autumn and winter than in those born in spring and summer at 30 d (Table 4). The CW value (20.5 cm) of calves born in spring was lowest at 60 d ($p = 0.001$). Also, the CW values were higher in calves born in summer and autumn than in those born in spring at 90 d ($p = 0.005$). Similarly, the calving season, statistically affected the WH values of calves at 60 d ($p = 0.001$), 90 d ($p = 0.002$), 120 d ($p = 0.032$), 150 d ($p = 0.040$). The calving season, statistically affected the RH values of calves at 60 d ($p < 0.001$), 120 d ($p = 0.004$), 150 d ($p = 0.009$). The BL value was highest in calves born in the summer months at 120 d ($p = 0.012$) and 150 d ($p < 0.001$). The BL value was higher in calves born in autumn than in those born in summer at 180 d. According to all the findings reported above, it is seen that the calving season affects the growth characteristics. Similarly, previous studies have reported that the calving season affects the LW values of calves at different age periods (Erdem et al., 2015; Alkoyak and Öz, 2022). The effects of the calving season on body characteristics can be explained by (i) their good use of pasture in spring and summer during the last days of pregnancy (Alkoyak and Öz, 2022), (ii) the difference in the vegetation period of the pasture (variation in the dry matter and other nutrient content of the pasture), (iii) and the change in the feeding profile in autumn and winter (variation in dry matter intake).

Tablo 4. Mean body traits of calves in different age periods according to calving season

Period	CS	n	LW	CG	CD	CW	WH	RH	BL
Birth	Autumn	27	33.8[ab]	72.4	26.2[a]	17.7	69.0	72.2	56.1
	Winter	16	35.5[b]	72.4	25.9[a]	17.3	69.8	73.4	54.6
	Spring	29	31.2[a]	70.1	26.8[ab]	17.8	69.2	72.3	54.2
	Summer	14	30.5[a]	70.0	28.2[b]	18.7	67.6	71.0	55.1
	SEM		0.66	0.47	0.25	0.21	0.50	0.53	0.42
	p-value		p = 0.048	p = 0.095	p = 0.028	p = 0.257	p = 0.600	p = 0.639	p = 0.335
30 d	Autumn	27	48.5	81.3	30.5[a]	21.3[b]	76.3	81.3	63.6
	Winter	16	52.1	82.7	31.1[a]	21.3[b]	78.1	82.9	62.9
	Spring	28	48.5	81.3	32.5[b]	20.1[a]	78.9	82.3	62.1
	Summer	14	52.8	84.9	33.7[b]	20.1[a]	80.4	83.9	65.6
	SEM		0.86	0.54	0.27	0.18	0.53	0.49	0.49
	p-value		p = 0.187	p = 0.094	p < 0.001	p = 0.014	p = 0.053	p = 0.367	p = 0.122
60 d	Autumn	27	61.6[a]	88.5[a]	33.3[a]	22.3[b]	82.1[a]	85.2[a]	66.3
	Winter	16	66.6[ab]	91.4[ab]	35.3[b]	21.9[b]	82.6[a]	87.1[a]	68.0
	Spring	28	61.15[a]	88.2[a]	34.7[ab]	20.5[a]	82.1[a]	85.9[a]	67.2
	Summer	13	72.0[b]	94.3[b]	37.0[c]	21.8[b]	87.3[b]	91.4[b]	70.1
	SEM		1.28	0.68	0.30	0.20	0.48	0.50	0.47
	p-value		p = 0.018	p = 0.011	p < 0.001	p = 0.001	p = 0.001	p < 0.001	p = 0.066
90 d	Autumn	25	74.3	94.1	36.4	23.4[b]	85.2[a]	89.8	69.1
	Winter	16	78.6	97.3	37.8	22.6[ab]	87.3[a]	90.8	72.3
	Spring	28	72.5	94.3	36.8	21.6[a]	85.9[a]	90.4	71.7
	Summer	13	80.7	98.4	38.5	23.1[b]	91.5[b]	94.5	71.9
	SEM		1.64	0.81	0.31	0.22	0.57	0.59	0.55
	p-value		p = 0.316	p = 0.199	p = 0.121	p = 0.005	p = 0.002	p = 0.061	p = 0.128

[a-c] Mean values in the same column with different superscripts differ ($p<0.05$); CS: Calving season; LW: Live weight; CG: Chest girth; CD: Chest depth; CW: Chest width; WH: Withers height; RH: Rump height; BL: Body length

Tablo 4. Cont.

Period	CS	n	LW	CG	CD	CW	WH	RH	BL
120 d	Autumn	24	87.1	100.1	38.1	24.1	88.4^{a}	92.3^{a}	72.3^{a}
	Winter	16	90.7	100.8	40.1	22.9	90.4^{ab}	94.0^{a}	74.3^{a}
	Spring	28	84.7	99.2	39.0	22.5	89.1^{a}	93.7^{a}	73.8^{a}
	Summer	13	95.9	105.8	40.6	22.7	93.1^{b}	98.4^{b}	78.3^{b}
	SEM		2.13	0.90	0.37	0.27	0.55	0.56	0.60
	p-value		p = 0.340	p = 0.098	p = 0.101	p = 0.096	p = 0.032	p = 0.004	p = 0.012
150 d	Autumn	24	100.0	105.8^{a}	41.1^{ab}	23.3	93.7^{a}	97.3^{a}	74.6^{a}
	Winter	16	103.0	106.7^{ab}	42.4^{bc}	24.5	93.1^{a}	97.5^{a}	76.9^{a}
	Spring	28	95.8	103.1^{a}	40.2^{a}	23.4	92.5^{a}	97.3^{a}	76.2^{a}
	Summer	13	113.6	111.8^{b}	43.4^{c}	23.7	97.5^{b}	102.6^{b}	82.3^{b}
	SEM		2.43	1.00	0.36	0.22	0.60	0.58	0.60
	p-value		p = 0.103	p = 0.036	p = 0.015	p = 0.274	p = 0.040	p = 0.009	p < 0.001
180 d	Autumn	24	112.0	111.5	43.0	24.1	96.5	100.0	78.2^{a}
	Winter	16	119.8	112.9	44.3	25.4	96.8	101.6	81.6^{ab}
	Spring	28	111.3	109.1	42.2	24.4	95.7	101.2	80.2^{ab}
	Summer	13	128.1	115.2	43.1	24.2	99.9	104.6	82.9^{b}
	SEM		3.01	1.15	0.41	0.27	0.62	0.65	0.59
	p-value		p = 0.235	p = 0.326	p = 0.341	p = 0.405	p = 0.316	p = 0.163	p = 0.043

[a-c] Mean values in the same column with different superscripts differ ($p<0.05$); LW: Live weight; CG: Chest girth; CD: Chest depth; CW: Chest width; WH: Withers height; RH: Rump height; BL: Body length

In the present study, the effects of the cow's parity on the LW ($p = 0.031$), CG ($p = 0.047$) and WH ($p = 0.030$) values of the calf at birth were remarkable findings (Table 5). At birth, the LW values of the calves of cows with parity of ≥5 (35.3 kg) were higher than the calves of cows with parity of 1 (30.4 kg). The results of the previous study on buffaloes were consistent with the findings of the present study (Uğurlu, et al., 2016; Kul et al., 2018). At the same period, the CG values of the calves of cows with parity of 4 (72.8 cm) and ≥5 (72.4 cm) were higher than the calves of cows with parity of 1 (68.6 cm). Similarly, the WH values of the calves of cows with parity of 4 (70.7 cm) and ≥5 (70.4 cm) were higher than the calves of cows with parity of 1 (66.2 cm). In summary, it was determined that as the parity of the cows increased, the LW, CG and WH values of the calves at birth increased. These results can be explained by changes in physiological factors that support the productive performance of buffalo cows as they reach their mature equivalents (do Nascimento Rangel et al., 2014).

Table 5. Mean body traits of calves in different growth periods according to parity

Period	Parity	n	LW	CG	CD	CW	WH	RH	BL
Birth	1	17	30.4[a]	68.6[a]	26.9	17.8	66.2[a]	69.8	53.3
	2	21	30.9[ab]	71.3[ab]	26.5	17.5	69.1[ab]	72.3	55.0
	3	13	32.2[abc]	71.2[ab]	26.6	17.6	68.3[ab]	71.6	54.2
	4	15	35.1[bc]	72.8[b]	26.1	17.6	70.7[b]	74.1	55.6
	5≤	20	35.3[c]	72.4[b]	26.3	18.5	70.4[b]	74.3	56.8
	SEM		0.66	0.47	0.25	0.21	0.50	0.53	0.42
	p-value		p = 0.031	p = 0.047	p = 0.645	p = 0.504	p = 0.030	p = 0.121	p = 0.080
30 d	1	16	49.0	81.9	32.4	19.4[a]	78.3	82.1	64.3
	2	21	48.7	81.6	32.1	20.4[ab]	77.2	80.7	61.7
	3	13	48.7	80.4	31.7	20.3[ab]	78.3	82.9	62.7
	4	15	51.9	83.2	31.3	21.2[b]	78.3	83.2	64.1
	5≤	20	51.0	83.2	31.4	21.5[b]	78.8	83.3	64.0
	SEM		0.86	0.54	0.27	0.18	0.53	0.49	0.49
	p-value		p = 0.683	p = 0.504	p = 0.672	p = 0.023	p = 0.895	p = 0.374	p = 0353
60 d	1	15	65.4	90.7	35.3	20.9	84.3	88.6	69.6
	2	21	60.9	88.5	34.4	21.3	81.5	85.9	67.9
	3	13	61.4	88.1	33.8	20.7	82.8	84.5	68.5
	4	15	67.9	91.5	35.4	22.2	83.8	88.3	68.1
	5≤	20	65.0	90.5	34.6	22.3	83.0	86.6	68.1
	SEM		1.28	0.68	0.30	0.20	0.48	0.50	0.47
	p-value		p = 0.401	p = 0.478	p = 0.509	p = 0.041	p = 0.386	p = 0.086	p = 0.521
90 d	1	15	78.1	97.1	37.3	22.0	88.4	91.2	72.9
	2	21	73.3	93.9	36.7	22.4	86.3	90.6	70.6
	3	12	68.5	92.8	36.7	22.3	86.2	89.9	69.8
	4	15	79.2	97.5	37.9	22.5	87.7	92.3	71.9
	5≤	19	77.5	96.0	37.1	23.4	86.0	90.8	70.2
	SEM		1.64	0.81	0.31	0.22	0.57	0.59	0.55
	p-value		p = 0.304	p = 0.323	p = 0.702	p = 0.307	p = 0.622	p = 0.837	p = 0.435

a-c Mean values in the same column with different superscripts differ ($p<0.05$); LW: Live weight; CG: Chest girth; CD: Chest depth; CW: Chest width; WH: Withers height; RH: Rump height; BL: Body length

Table 5. Cont.

Period	Parity	n	LW	CG	CD	CW	WH	RH	BL
120 d	1	15	92.5	101.3	39.5	22.2	90.4	95.7	77.3
	2	20	87.2	99.8	39.5	23.2	89.5	93.6	72.2
	3	12	78.0	97.3	37.8	22.6	89.1	92.6	72.8
	4	15	93.0	103.5	40.3	23.7	91.3	94.6	74.3
	5≤	19	89.3	101.7	38.8	23.6	89.0	94.0	74.7
	SEM		2.13	0.90	0.37	0.27	0.55	0.56	0.60
	p-value		p = 0. 268	p = 0.351	p = 0.332	p = 0.372	p = 0.668	p = 0.575	p = 0.053
150 d	1	15	107.1	106.9	41.4	23.0	95.7	99.9	79.5
	2	20	102.0	104.6	41.3	23.4	93.5	98.2	75.9
	3	12	86.5	100.7	39.5	23.3	90.7	95.0	74.8
	4	15	106.1	109.1	42.5	24.5	94.9	99.3	77.9
	5≤	19	101.4	107.6	41.4	24.0	93.6	97.9	76.2
	SEM		2.43	1.00	0.36	0.22	0.60	0.58	0.60
	p-value		p = 0. 116	p = 0.116	p = 0.181	p = 0.200	p = 0.146	p = 0.142	p = 0.146
180 d	1	15	123.5	112.3	42.3	23.7	98.1	103.4	82.2
	2	20	117.5	110.8	42.8	24.7	98.0	102.5	79.6
	3	12	97.6	104.6	41.5	23.8	93.3	97.3	77.8
	4	15	122.2	115.3	45.0	25.3	97.4	102.3	80.6
	5≤	19	114.7	113.2	43.2	24.5	96.3	100.9	80.9
	SEM		3.01	1.15	0.41	0.27	0.62	0.65	0.59
	p-value		p = 0. 104	p = 0.085	p = 0.119	p = 0.380	p = 0.149	p = 0.061	p = 0.257

LW: Live weight; CG: Chest girth; CD: Chest depth; CW: Chest width; WH: Withers height; RH: Rump height; BL: Body length

CONCLUSIONS

It was concluded that the environmental factors examined affected the LW and body traits of calves at different growth periods. Controlling the negative effects of these environmental factors by improving rearing and feeding conditions is important in terms of increasing the growth performance of buffalo calves. Eliminating the effects of these environmental factors will increase the success rate of future selection programs aimed at improving the growth performance of buffalo calves.

ACKNOWLEDGMENTS

The authors thank the Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies, Ondokuz Mayıs University and the Samsun Provincial Buffalo Breeders Association for supporting and contributing to this research. This study was supported by Ondokuz Mayıs University (Project no: PYO. ZRT.1901.19.003).

CONFLICTS OF INTEREST

The authors declare no conflict of interest

REFERENCES

Akdağ, A., Okuyucu, İ. C., Erdem, H., Kul, E., Ocak, N. 2024. Some Welfare Assessment Traits and Quantitative-Qualitative Milk Parameters as Affected by Supplementary Feeding at Milking and Parity in Anatolian Buffalo Cows. Animals, 14(6), 956.

Alkoyak, K., Öz, S. 2022. The effect of nongenetic factors on calf birth weight and growth performance in Anatolian buffaloes. Turkish Journal of Veterinary & Animal Sciences, 46(4), 609-616.

Barbosa, S.B.P., Lopes, C.R.A., Pereira, R.G.A., Santoro, K.R., López, O.M., Rezende, F.M. 2006. Environmental and inherited factors as sources of variation in buffalo birth weight. In 8th World Congress on Genetic Applied to Livestock Production, August (pp. 13-18).

Bezdíček, J., Nesvadbová, A., Makarevich, A., Kubovičová, E. 2020. Relationship between the animal body condition and reproduction: the biotechnological aspects. Archives Animal Breeding, 63(1), 203-209.

Çelikeloğlu, K., Erdoğan, M., Koçak, S., Zemheri, F., Tekerli, M. 2015. The effect of environmental factors and growth hormone receptor gene polymorphism on growth curve and live weight parameters in buffalo calves. Lalahan Hayvancılık Araştırma Dergisi, 55 (2): 45-49.

do Nascimento Rangel, A.H., de Oliveira, J.P.F., de Medeiros, H.R., de Araújo, V.M., Novaes, L.P., de Lima Júnior, D.M. 2014. Influence of Murrah buffalo behavior in milking parlors on production characteristics. Arch. Vet. Sci. 19 (3), 53–61.

El-Bramony, M.M., Fooda, T.A., & Gebreel, I.A. 2008. Estimation of direct genetic and maternal effects for weaning traits in Egyptian buffalo. Egyptian Journal of Animal Production, 45, 1-9.

Erdem, H., Atasever, H., Kul, E., Önder, H., Demirci, H. 2015. Growth characteristics of Anatolian buffalo calves reared in farm conditions: A case study in Samsun province of Turkey. 8th Asian Buffalo Congress, p:25 April 21-25, Istanbul, Turkey.

Erdem, H., Okuyucu, I. C., Demirci, H. 2022. Components and specific gravity of colostrum from Anatolian buffalo cows and effects on growth of buffalo calves. South African Journal of Animal Science, 52(3), 316-325.

Hossein-Zadeh, N. G., Madad, M., Shadparvar, A. A., Kianzad, D. 2012. An observational analysis of secondary sex ratio, stillbirth and birth weight in Iranian buffaloes (Bubalus Bubalis). Journal of Agricultural Science and Technology, 14, 1477-1484.

Kharkar, K.P., Raghuwanshi, D.S., Thakre, P.D., Lende, S.R. Khati, B.M. 2019. Effect of non-genetic parameters on mortality pattern in Nagpuri buffalo calves. Journal of Animal Health Production, 7(1), 1-4.

Kul, E., Filik, G., Şahin, A., Çayıroğlu, H., Uğurlutepe, E., Erdem, H. 2018. Effects of some environmental factors on birth weight of Anatolian buffalo calves. Turkish Journal of Agriculture-Food Science and Technology, 6(4), 444-446.

Marai, I. F. M., Farghaly, H. M., Nasr, A. A., Abou-Fandoud, E. I., Mohamed, I. A. S. 2001. Buffalo cow productive, reproductive and udder traits and stayability under sub-tropical environmental conditions of Egypt. Journal of Agriculture in the Tropics and Subtropics, 102(1), 1-14.

Metin Kıyıcı, J., Sarıaslan, Y. 2023. Holstein Irkı Gebe Düvelerde Vücut Kondisyon Skoru ile Süt Verimi, Buzağı Performansı ve Gebelik İlişkilerinin Araştırılması. Journal of the Institute of Science & Technology/Fen Bilimleri Estitüsü Dergisi, 13(3). [In Turkish].

Okuyucu İ. C., Erdem H. 2018. Non-genetic factors affectıng colostrum quality and effects of barning type and colostrum qualıty on growth performance of Holsteın calves. Academia Journal of Engineering and Applied Sciences, 1(4), 47-60.

Singh, R., Randhawa, S.S., Randhawa, C.S. 2017. Body condition scoring by visual and digital methods and its correlation with ultrasonographic back fat thickness in transition buffaloes. Buffalo Bulletin, 36(1), 169-182.

Sorathiya, L. M., Fulsoundar, A. B., Kharadi, V. B. 2009. Environmental and genetic effects on body weight in Surti buffalo calves. Indian Journal of Animal Sciences, 79(11), 1176.

Şekerden, Ö. 2010. Anadolu ve Anadolu x İtalyan melezi manda buzağılarının büyüme özellikleri ve bunlar üzerine genotip, cinsiyet ve doğum yılı etkileri. *Hayvansal Üretim*, *51*(2), 34-43. [In Turkish].

Şekerden, Ö., Tapkı, İ. 2003. Hatay İli Anadolu mandalarında köy şartlarında büyüme özellikleri. Atatürk Üniv. Zir. Fak. Derg. 34(1): 51-55. [In Turkish].

Uğurlu M, Kaya İ, Saray M. (2016). Effects of some environmental factors on calf birth weight and milk yield of Anatolian Water Buffalo (Bubalus bubalis). Bulgarian Journal of Agricultural Science; 22 (6): 995-998.

DETERMINATION OF PHOSPHORUS, ZINC AND IRON FERTILIZER NEEDS OF SIYEZ WHEAT (*Triticum monococcum* L.) GROWN IN CALCAREOUS SOIL OF KONYA CITY

Fırat UZUN[1] *AYŞEN AKAY*[2]

[1,2]University of Selcuk, Faculty of Agricultural, Department of Soil Science and Plant Nutrition,42130, Konya, Türkiye

Corresponding author email: aakay@selcuk.edu.tr

ABSTRACT

In ancient times, wheat was first cultivated on the hills above the Tigris river, and it is known that emmer and siyez wheat were among the first crops grown in this region. The history of Siyez wheat used in this study dates back to 12,000 years ago, according to some sources. In Turkey, it is grown with traditional agricultural methods in narrow areas, especially around Kastamonu, Sinop and to a limited extent in the provinces of Çankırı, Çorum, Bilecik, Kayseri, Erzincan, Tekirdağ, Kocaeli, Edirne and Kars. Studies on the nutrient requirements of Siyez wheat are insufficient. The aim of this study was to determine the iron, zinc and phosphorus fertilizer requirements of the crop during its cultivation on calcareous soils. The experiment was carried out under greenhouse conditions with different doses of phosphorus (0 and 4 kg P_2O_5 da^{-1}), zinc (0, 4, 8, 16 mg Zn kg^{-1}) and iron (0, 4, 8, 16 mg Fe kg^{-1}). According to the results obtained, the average plant height was between 82.67-102.44 cm, the number of spikelets was 21.33-29.00 p/pod, the length of spikelets was 2.90-4.03 cm, the biomass yield was 18.42-25.08 g/pod and the grain yield was 4.55-6.47 g/pod. When grain yield (g/pot) was evaluated, there was a significant increase in yield with the P0Fe16Zn4, P0Fe4Zn8, P1Fe8Zn4 and P1Fe0Zn16 treatments. Leaf and grain Fe concentrations ranged between 33.9-88.7 and 24.3-55.1 mg kg^{-1}, leaf and grain Zn concentrations ranged between 31.4-69.8 and 48.9-109.9 mg kg^{-1}, leaf and grain P concentrations ranged between 0.13-0.20 and 0.27-0.39%, leaf and grain K concentrations ranged between 0.54-0.66 and 0.38-0.42%. According to the statistical analyses, significant differences were observed between Fe and Zn application doses ($P<0.01$). The Fe*Zn combination was also statistically significant at $p<0.01$ level. According to the results of the study, if there is sufficient amount of phosphorus in the soil, 1 kg Fe /da and 2 kg Zn /da application can be recommended and if there is insufficient amount of phosphorus in the soil, 4 kg P /da, 2 kg Fe /da and 1 kg Zn /da application can be recommended.

Keywords: Zinc, iron, phosphorus, calcareous soil, siyez(einkorn) wheat

INTRODUCTION

One of the most important interventions to minimize nutrient deficiencies in humans in developing countries is the selection and development of plant genotypes that contain high concentrations of micronutrients in the edible parts. At present, half of the world's population

is suffering from micronutrient deficiencies, especially Fe and Zn (Welch and Graham, 1999). Zn and Fe deficiency causes serious health problems such as immune system disorders, poor physical growth, impaired mental and cognitive development, and anemia (Welch and Graham, 1999; Hotz and Brown, 2004). Developing countries widely consume cereal-based foods as their primary source of calories. However, the concentration and bioavailability of Zn and Fe in cereal seeds are naturally low. Welch and Graham (1999) reported that cereal grains are the major source of Fe and Zn for people in developing countries, but the intakes do not satisfy their mineral demands. Therefore, an increase in the total amount and bioavailability of Zn and Fe in food crops is an important necessity. Due to the high prevalence of Fe deficiency in humans, increasing the concentration of Fe in food is a major global concern (Aciksoz et al., 2011). Iron deficiency is the most common cause of anemia throughout the world. According to a recent report based on the WHO database, approximately 1.6 billion people are affected by anemia (McLeon et al., 2009). The improvement of both iron concentration and bioavailability in cereal grains is therefore a major challenge and a high priority research area (Cakmak et al., 2010). Due to the high rate of decomposition of Fe into unavailable forms when applied to calcareous soils and the low mobility of Fe in the phloem, soil and/or foliar Fe fertilization is less effective than Zn fertilization for grain enrichment (Cakmak, 2008). Zinc deficiency has been identified as a major risk factor for human health worldwide. According to the World Health Organization (WHO, 2002) report on the risk factors responsible for the development of disease and illness, "Zn deficiency" is ranked 11th among the top 20 most important disease factors (Hotz and Brown, 2004).
In this study, compared to wild and cultivated wheat varieties such as *T. dicoccon* and *T. dicoccoides*, *Triticum monococcum* was found to be a much more promising genetic donor for micronutrients (Cakmak et al., 2000). In recent years, the trend toward low-impact and sustainable agriculture, coupled with increasing interest in the nutritional components of food, has led to the rediscovery of many forgotten crops, including siyez (einkorn). Today, traditional einkorn is cultivated in mountainous regions of Turkey, the Balkans, southern Italy, southern France, Spain, and Morocco. However, yields are relatively low compared to bread wheat varieties (Loje et al., 2003). In Turkey, einkorn (Iza/Siyez) and emmer (kaplıca/chatalsiyez/gernik/kavılca) are still grown in limited areas in the poor highlands of the Western Black Sea region, which extends from the western end of the Kızılırmak delta to the east of Adapazarı and Bilecik, reaching 2000 m above sea level in the west (Kan et al., 2015). Siyez (T. *monococcum*) is a diploid species. It derives from its wild relative, wild siyez (*T. baeoticum*). Southeast Anatolia is believed to have first grown it (Diamond, 1997; Heun et al., 1997; Nesbit and Samuel, 1998; Lev-Yadun et al., 2000). Siyez common wheat, also known as einkorn wheat (*Triticum monococcum ssp. monococcum* L.) in foreign sources, is suitable for organic farming, and compared to bread wheat, it is often more disease-resistant and drought-tolerant. It is also rich in starch, fibers, minerals, and secondary plant compounds (Arzani et al., 2017; Nesbitt and Samuel, 1996). It has been reported that up to 30% of the world's total land area is composed of calcareous soils, the availability of iron in the soil is significantly reduced as a result of precipitation of inorganic Fe^{+3} due to high soil pH, and iron deficiency inhibits the growth and yield of most crops in calcareous soils (Ohwaki et al., 1997); it has been reported that about 50% of agricultural soils in our country are deficient in Zn (Eyüpoğlu et al., 1995), and this rate reaches up to 90%, especially in the region of Konya (Cakmak et al., 1996).

The aim of this study was to determine the appropriate fertilizer doses for Siyez wheat cultivation in Konya calcareous soils and increase grain Zn and Fe concentrations.

MATERIALS AND METHODS

In the study, siyez wheat obtained from the Agricultural Credit Cooperative of İhsangazi district of Kastamonu province as experimental plants and nutrient-poor calcareous soil were used. Four doses of iron (0-4-8-16 mg kg^{-1} Fe; Fe-EDDHA (6% Fe) form) and four doses of zinc (0-4-8-16 mg kg^{-1} Zn¸ $ZnSO_4.7H_2O$ form) were applied to the experimental plants. In a study conducted in a total of 64 pots with four replications under greenhouse conditions according to a randomized factorial plot experimental design. In addition, 4 kg N da^{-1} (in the form of AN (33%), and 2.5 kg K da^{-1} (in the form of K_2SO_4 (51% K_2O) fertilizers were applied to all pots before planting. The experimental soil was collected from a depth of 0–30 cm, sieved through a 4 mm sieve, and filled into the pots by weighing (7 kg of soil pot^{-1}) as an oven-dried basis. 15 wheat seeds were planted in each pot. After 15 days of emergence, the plants were thinned out, leaving 10 plants in each pot. The pots were watered daily and the soil was irrigated to keep the soil at field capacity, and the pot placement was changed regularly every week so that the pots could benefit from temperature and sunlight homogeneity. Flag leaf samples were collected from all plants at the end of the 6-month growth period, and these leaves were stored for elemental analysis after preliminary preparation. The Siyez wheat plants were harvested at the end of the 227-day (7.5-month) growth period, after grain formation and ripening were completed. Wet and dry plant weights and grain weights of plants in each pot were determined individually. Leaf samples and above-ground parts of harvested plants were dried in an oven at 65^0C for 48 hours, weighed, and then ground. Flag leaf samples taken from the pots during the heading period and grain samples taken at harvest time were subjected to wet digestion with H_2SO_4 and H_2O_2 after pre -treatment (Kacar and Inal, 2010). The contents of P, K, Zn, and Fe (Bayraklı, 1987) were determined in the obtained filtrates. In the calculation of crude protein in the grain, the nitrogen value in the grain was calculated by multiplying it by a factor of 6.25. In addition, the soil used in the pot experiment was analyzed for fertility, and the results are presented in Table 1.

Statistical analysis of the values obtained as a result of the research, it was carried out with the Minitab 18 Statistical Software package program. All data (Fe, Zn, and Fe*Zn interactions) were subjected to the Tukey's multiple comparison test to determine the statistical significance of the treatment effects. Group means were compared using the least significant difference option with $p \leq 1\%$ and $p \leq 5\%$, as shown in the tables with the lettering.

Table 1. The analysis results of experiment soil

Parameters	Results	Commentary	Literature of analysis method
pH (1:2.5 soil:water)	7.45	Neutral	(Richards, 1954)
EC (1:2.5 soil:water) (µS cm^{-1})	208.74	Lightly Salt	(Richards, 1954)
$CaCO_3$(%)	38.23	High	(Bayraklı,1986)
Organic matter (%)	2.03	Poor	(Walkley and Black, 1934)
Clay(%)	58.17		
Silt(%)	16.75		
Sand(%)	25.08		
Texture class	Clay		(Gee and Bauder, 1986)
Ca(mg kg^{-1})	8510.4	Excess	(Thomas,1982)
Mg(mg kg^{-1})	329.14	Sufficient	(Thomas,1982)
K(mg kg^{-1})	197.78	Sufficient	(Carson,1980)
Na(mg kg^{-1})	25.38		(Thomas,1982)
P(mg kg^{-1})		Sufficient	(Olsen and Sommers, 1982)
Fe(mg kg^{-1})	1.12	Deficiency	
Zn(mg kg^{-1})	0.31	Deficiency	(Lindsay and Norvell, 1978)
Mn(mg kg^{-1})	3.54	Deficiency	
Cu(mg kg^{-1})	0.84	Sufficient	

RESULTS AND DISCUSSIONS

According to the results of the analysis of variance on the effects of different doses of iron and zinc fertilizers applied on grain and leaf N, P, K, Fe, and Zn concentrations of Siyez wheat, applied Fe doses showed a significant effect on leaf N, K, Fe, Zn concentrations and grain P, Fe, Zn concentrations ($p<0.01$) (Table 2). The applied Zn doses showed a significant statistical effect on leaf N, Fe, and Zn concentrations and grain N, protein, and K, Zn concentrations ($p<0.01$). When the Fe*Zn interaction was analyzed, the effect on the concentrations of N, K, Fe, Zn in the leaves and N, protein, Fe, Zn in the grain was also statistically significant at the $p<0.01$ level.

When the mean values of fertilizer applications were examined (Table 3), increasing doses of iron significantly increased grain Fe, Zn, and leaf K and Fe concentrations compared to the control ($p<0.01$). However, it significantly and negatively affected grain P concentration compared to the control ($p<0.01$). In general, the Fe16 dose showed the highest values among these parameters. Iron fertilization (Table 3) had a positive effect on grain micronutrient concentration. K, Zn, leaf Zn, and Fe concentrations were significantly and positively affected by increasing zinc fertilization ($p<0.01$). Zinc fertilization significantly increased the grain's Zn concentration. Grain Zn concentration increased significantly compared to the control, particularly at the Zn16 (89.91 mg kg^{-1}) dose. The Zn8 (0.42%) dose had the highest K content in grains ($p<0.01$).

The leaf and grain N concentrations of Siyez wheat showed significant differences with the combined effect of Fe and Zn treatments. The grain N concentration ranged from 1.58 to 1.83%, while the leaf N concentration ranged from 0.45 to 0.76% (Figure 1). The values of grain protein content varied between 10.44 and 11.14% (Table 3). According to a study, the average value of protein content in the grain was 14.4% (Atar and Kara, 2017). In their study, which also used siyez wheat under Canadian conditions, Dorval et al. (2015) reported that the protein content of all varieties varied between 14.2 and 15.4%. Erdoğan (2023) studied siyez wheat grown in Bursa with different N doses and found that the average protein content ranged from 9.51 to 11.37 percent. The highest values were found with N doses of 20, 10, and 15 kg N/ha. Another study reported a variation in the protein content of siyez wheat from 13.49 to 19.01% (Demirel, 2013).

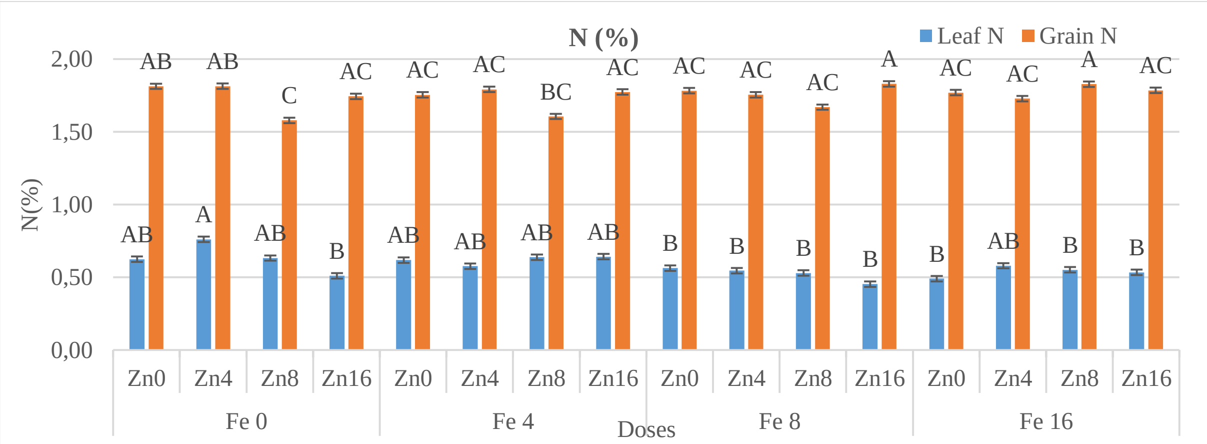


Figure 1. Effect of Fe and Zn applications on grain and leaf N concentration (%) of siyez wheat (Different letters at the columns show significant differences at $p < 0.01$ for applications).

The addition of Fe and Zn to Siyez wheat (*Triticum monococcum*) resulted in a change in the grain P concentration from 0.27 to 0.39% and the leaf P concentration from 0.13 to 0.20% (Figure 2). We observed the highest grain P concentration at the Fe0Zn8 (0.39%) and Fe0Zn16 (0.37%) doses, and the highest leaf P concentration at the Fe0Zn0 (0.19%) and Fe4Zn8 (0.20%) doses. When the results are compared with the literature, the average amount of P in Siyez wheat samples was 0.52% (Suchowilska et al., 2012), the average amount of P in grains was 0.54% (Erba et al., 2011), and the average P in grain was 0.48% (Zengin, 2015), which was in the range of 0.35-0.63%. Furthermore, Zencirci et al. (2021) found the highest concentration of P to be 5687 mg/kg.

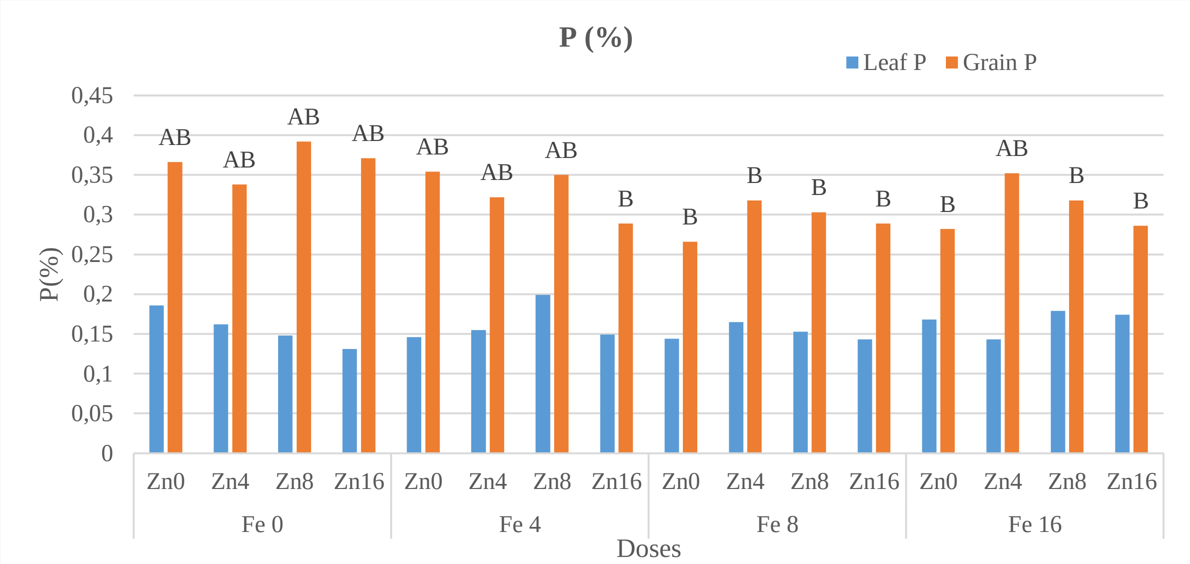


Figure 2. Effect of Fe and Zn applications on grain and leaf P concentration (%) of siyez wheat (Different letters at the columns show significant differences at $p < 0.01$ for applications)

The grain K concentration was lowest at Fe8Zn0 (0.38%) and highest at Fe0Zn8 (0.42%) (Figure 3). The leaf K concentration ranged from 0.54 to 0.66%, with the Fe16Zn8 (0.66%) and Fe16Zn16 (0.65%) doses exhibiting the highest K concentration. When the results were compared with the literature, the average K concentration of Siyez wheat was 0.39% (Abdell-All et al., 1995); in another study it ranged from 0.39 to 0.47% with an average of 0.43% (Suchowilska et al., 2012), and the highest K concentration (565 mg/kg) was found in Siyez grains from Kastamonu İhsangazi (population 4) (Zencirci et al., 2021). The highest potassium content in siyez wheat treated with biochar was 2.45% (Çığ et al., 2021), and in another study it was reported to vary between 0.22-0.62% with an average of 0.46% (Zengin, 2015).

Fe treatments ($p<0.01$) and Fe*Zn interaction ($p<0.01$) had a statistically significant effect on grain Fe concentration, while Fe and Zn treatments ($p<0.01$) and Fe*Zn interaction ($p<0.01$) had a statistically significant effect on leaf Fe concentration (Figure 4). The lowest Fe concentration in the grain was 24.3 mg kg^{-1} at the Fe0Zn16 dose, while the highest value was 55.1 mg kg^{-1} at the Fe16 Zn4 dose. Leaf Fe concentration ranged from 33.9 to 88.7 mg kg^{-1}. The highest leaf Fe concentrations were observed at Fe16Zn8 (88.73 mg kg^{-1}) and Fe16Zn16 (63.98 mg kg^{-1}).

Table 2. The results of variance analysis of the effect of Fe and Zn fertilizer applications on the concentration of various plant nutrients of siyez wheat (* P <0.05, ** P <0.01)

Applications	SD	Grain N (%)	Leaf N(%)	Grain Protein (%)	Grain P (%)	Leaf P (%)	Grain K (%)	Leaf K (%)	Grain Fe (mg kg^{-1})	Leaf Fe (mg kg^{-1})	Grain Zn (mg kg^{1})	Leaf Zn (mg kg^{1})
	F values											
Fe	3	-	**	-	**	-	-	**	**	**	**	*
Zn	3	**	**	**	-	-	**	-	-	**	**	**
Fe*Zn	9	**	**	**	-	-	-	**	**	**	**	**
Error	48											
Total	63											

Table 3. The effect of Fe and Zn fertilizer applications on various plant nutrient concentrations of siyez wheat (P <0.01)

Fe Dozları *Fe doses* *(mg kg^{-1})*	Tane N(%) *Grain N(%)*	Yaprak N(%) *Leaf N(%)*	Tane Protein (%) *Grain Protein (%)*	Tane P(%) *Grain P (%)*	Yaprak P(%) *Leaf P(%)*	Tane K (%) *Grain K (%)*	Yaprak K (%) *Leaf K (%)*	Tane Fe (mg kg^{-1}) *Grain Fe (mg kg^{-1})*	Yaprak Fe (mg kg^{-1}) *Leaf Fe (mg kg^{-1})*	Tane Zn (mg kg^{-1}) *Grain Zn (mg kg^{-1})*	Yaprak Zn (mg kg^{-1}) *Leaf Zn (mg kg^{-1})*
0	1,74± 0,12	0,63± 0,11A	10,86± 0,74	0,37± 0,05A	0,16± 0,03	0,41± 0,02	0,57± 0,04B	26,15± 4,84B	53,82± 9,81A	65,38± 8,18C	48,09± 6,49
4	1,73± 0,10	0,62± 0,05A	10,82± 0,63	0,33± 0,05AB	0,16± 0,04	0,41± 0,02	0,59± 0,04AB	35,30± 13,89AB	38,30± 11,04B	69,82± 11,33BC	51,43± 6,72
8	1,76± 0,09	0,52± 0,07B	11,00± 0,58	0,29± 0,04B	0,15± 0,03	0,41± 0,02	0,59± 0,02AB	33,67± 8,94AB	48,21± 9,08AB	74,43± 16,35B	48,63± 5,900
16	1,78± 0,07	0,54± 0,08B	11,11± 0,42	0,31± 0,05B	0,17± 0,02	0,42± 0,02	0,63± 0,05A	44,51± 11,86A	58,04± 25,83A	87,85± 19,82A	51,87± 17,50
Zn Dozları *Zn doses* *(mg kg $^{-1}$)*											
0	1,78± 0,06A	0,57± 0,08AB	11,12± 0,380A	0,32± 0,06	0,16± 0,04	0,40± 0,02B	0,60± 0,02	35,07± 8,27	44,11± 11,62B	55,14± 5,48C	39,80± 6,18C
4	1,77± 0,06A	0,62± 0,11A	11,08± 0,373A	0,33± 0,04	0,16± 0,03	0,41± 0,02AB	0,60± 0,04	35,01± 15,69	43,15± 10,23B	74,39± 8,71B	47,99± 7,01B
8	1,67± 0,12B	0,59± 0,07AB	10,44± 0,726B	0,34± 0,05	0,17± 0,04	0,42± 0,01A	0,59± 0,05	34,04± 10,67	60,33± 24,40A	78,03± 12,79B	54,48± ,74A
16	1,78± 0,09A	0,54± 0,09B	11,14± 0,567A	0,31± 0,07	0,15± 0,02	0,41± 0,02AB	0,59± 0,05	35,52± 13,76	50,78± 12,84AB	89,91± 14,88A	57,75± 6,07A

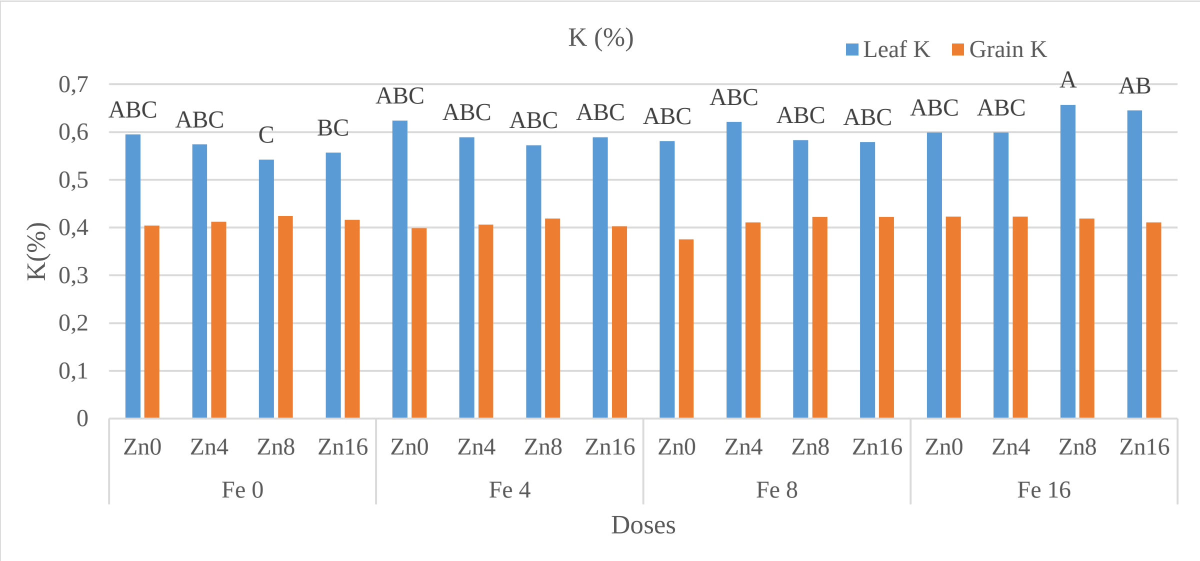


Figure 3. Effect of Fe and Zn applications on grain and leaf K concentration (%) of siyez wheat (Different letters at the columns show significant differences at $p < 0.01$ for applications)

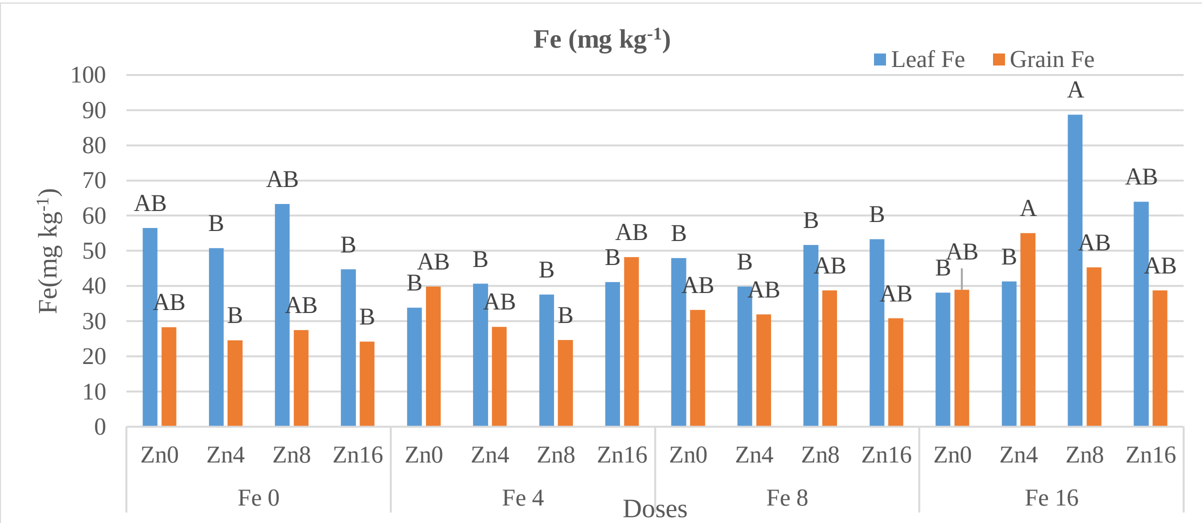


Figure 4. Effect of Fe and Zn applications on grain and leaf Fe (mg kg^{-1}) concentration of siyez wheat (Different letters at the columns show significant differences at $p < 0.01$ for applications)

There were statistically significant changes in the amount of Fe in the grains ($p<0.01$) and the amount of Fe in the leaves ($p<0.01$) after Fe treatments ($p<0.01$). There were also statistically significant changes in the amount of Fe in the grains and leaves ($p<0.01$) at Fe*Zn interaction(Figure 4). The lowest Fe concentration in the grain was 24.3 mg kg^{-1} at the Fe0Zn16 dose, while the highest value was 55.1 mg kg^{-1} at the Fe16 Zn4 dose. Leaf

Fe concentrations ranged from 33.9 to 88.7 mg kg^{-1}, with the highest observed at Fe16Zn8 (88.73 mg kg^{-1}) and Fe16Zn16 (63.98 mg kg^{-1}).

When the results compare with the findings of previous studies on *Triticum monococcum* wheat, Suchowilska et al. (2012) reported that the Fe concentration of T. *monococcum* wheat varied between 32 and 62 mg kg^{-1}, Cakmak et al. (2000) reported that the Fe concentration in T. *monococcum* wheat samples varied between 34 and 85 mg kg^{-1}. Erba et al. (2011) reported an average of 52.3 mg kg^{-1}, and Zengin (2015) reported that grain Fe concentration was between 24.19-44.43 mg kg^{-1}. Fe content in 54 different siyez samples grown in the Adana region in 2003-2004 in the mountainous and plain areas was found to be between 32 mg kg^{-1} and 61 mg kg^{-1} in the mountainous area and between 35 mg kg^{-1} and 85 mg kg^{-1} in the plain area (Ozkan et al., 2007). In addition, Siyez wheat from Kastamonu İhsangazi (Population-4) was reported to have the highest Fe content (59.32 mg kg^{-1}) (Zencirci et al., 2021).

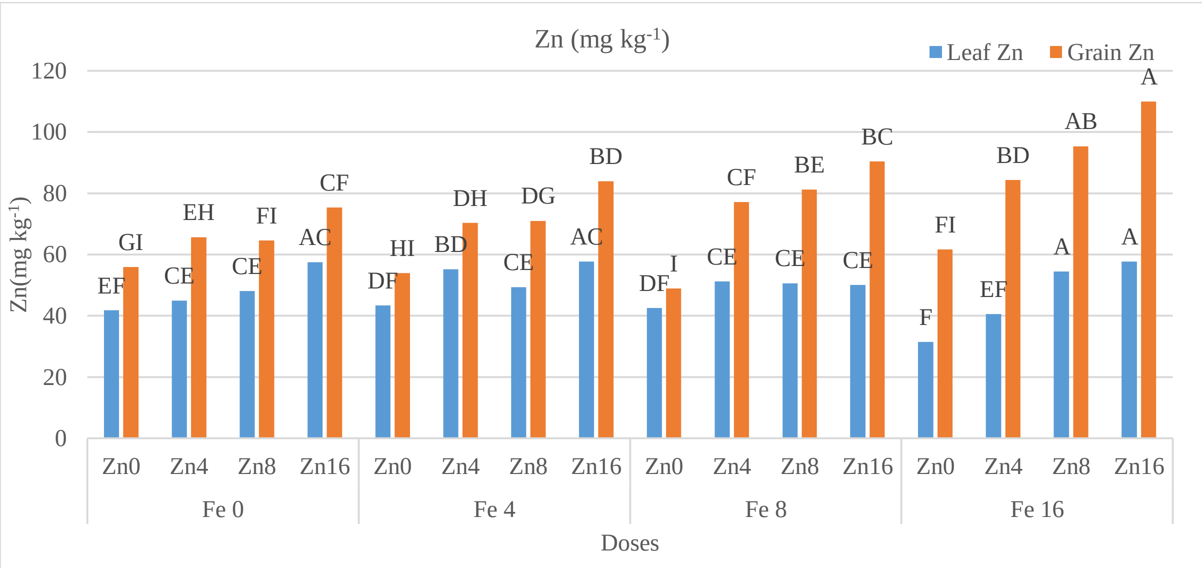


Figure 5. Effect of Fe and Zn applications on grain and leaf Zn (mg kg^{-1}) concentration of siyez wheat (Different letters at the columns show significant differences at $p < 0.01$ for applications)

The effects of Fe, Zn treatments, and Fe*Zn interaction ($p<0.01$) on the Zn concentration in the grain and leaves were found to be statistically significant (Figure 5). The lowest grain Zn concentration was 48.9 mg kg^{-1} at the dose of Fe8 Zn0, while the highest value was 110.0 mg kg^{-1} at the dose of Fe16 Zn16. In general, as Fe and Zn doses increased, the concentration of Zn in the grain increased significantly. Leaf Zn concentration varied between 31.5-57.8 mg kg^{-1} and the highest leaf Zn concentrations were observed at Fe16Zn8 (69.84 mg kg^{-1}) and Fe16Zn16 (65.62 mg kg^{-1}) doses. According to various studies, the amounts of Zn reported by Ozkan et al. (2007) for 54 different *Triticum monococcum* wheat cultivars were between 36 mg kg^{-1} and 76 mg kg^{-1} in dry matter.

Suchowilska et al. (2012) reported Zn contents of 33-68 mg kg^{-1} in 12 different siyez wheats. Cakmak et al. (2000) reported 29-89 mg kg^{-1} in 13 different siyez varieties, Bálint et al. (2001) reported 33.9-41.8 mg $kg^{-1,}$ Gabrovska et al. (2002) had an average of 42 mg kg^{-1}, Erba et al. (2011) 59.6-87.4 mg kg^{-1}, Zencirci et al. (2021) had the highest 74.68 mg kg^{-1}, and Zhao et al. (2009) reported that it was between 20.1-27.8 mg kg^{-1}. These values are parallel and even higher than the data from our research. The Zn concentration in the grain increased with Zn fertilizer application. It was observed that the average leaf Zn contents obtained in our study were sufficient considering the limits established for the concentration of Zn (mg kg^{-1}) in the vegetative parts of wheat plants (deficient <15, sufficient 15-70, and excess >70 mg kg^{-1}) (Ibrikçi et al., 1994).

CONCLUSION

Since the exact amounts of pure macro and micronutrients used in the production of siyez wheat in our country are not available in different sources, in this study it was found that applying Fe and Zn fertilizers to siyez wheat had positive results both in determining the macro element concentration of the product and in increasing the micronutrient concentrations. The application of increasing doses of Fe and Zn fertilizers significantly increased the grain potassium and grain zinc concentrations. In the case of Fe and Zn deficiency in the soil, we recommend applying 1 kg Fe da^{-1} and 2 kg Zn da^{-1} fertilizer for siyez growth.

ACKNOWLEDGEMENTS

The BAP coordination office of Selçuk University has supported this study under research project number 18401120. It is also a result of the MSc . thesis of the first author.

REFERENCES

Abdel-All, E., Hucl, P. Sosulski, F. 1995. Compositional and Nutritional Characteristics of Spring Einkorn and Spelt wheats, *Cereal Chemistry (USA)*.

Aciksoz, SB., Yazici, A. Ozturk, L. Cakmak, I. 2011. Biofortification of wheat with iron through soil and foliar application of nitrogen and iron fertilizers. *Plant and Soil*, *349*, 215-225.

Atar, B., Kara, B. 2017. Comparison of Grain Yield and Some Characteristics of Hulled, Durum and Bread Wheat Genotypes Varieties, *Turkish Journal of Agriculture-Food Science and Technology*, 5 (2), 159-163.

Arzani, A., Ashraf, M. 2017. Cultivated ancient wheats (Triticum spp.): A potential source of health-beneficial food products. Comparative Review of Food Science and Food Safety, 16, 477-488.

Bálint, AF., Kovács, G. Erdei, L. Sutk, J. 2001. Comparison of the Cu, Zn, Fe, Ca and Mg Contents of the Grains of Wild, Ancient and Cultivated Wheat Species, Cereal Research Communications, 375-382.

Bayraklı, F. 1986. Toprak ve Bitki Analizleri (Çeviri). 19 Mayıs Üniversitesi Ziraat Fakültesi S:77-79, Samsun.

Cakmak, I., Yilmaz, A. Kalayci, M. Ekiz, H. Torun, B. Ereno, B. Braun, H. 1996. Zinc Deficiency as a Critical Problem in Wheat Production in Central Anatolia, *Plant and Soil*, 180 (2), 165-172.

Cakmak, I., Ozkan, H. Braun, H. Welch, R. Romheld, V. 2000. Zinc and Iron Concentrations in Seeds of Wild, Primitive, and Modern Wheats, Food and Nutrition Bulletin, 21 (4), 401-403.

Cakmak, I. 2008. Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil 302:1–17

Cakmak, I., Pfeiffer, WH. McClafferty, B. 2010a. Biofortification of durum wheat with zinc and iron. Cereal Chem 87:10– 20

Carson, PL., 1980. Recommended Potasium Test. Recommended Potasium test. In: Recommended Chemicalsoil Test Procedures for the North Central Region. Rev.ed., North Central Regional Publication no.221. North Dakota Agric. Exp. Stn. North Dakota StateUniversity, Fargo. (USA).

Çığ, F., Sönmez, F. Nadeem, MA. Sabagh, AE. 2021. Effect of biochar and PGPR on the growth and nutrients content of einkorn wheat (Triticum monococcum L.) and post-harvest soil properties. *Agronomy*, *11*(12), 2418.

Diamond, J., 1997. Location, Location, Location: The First Farmers. Science, 278, 1243–1244.

Dorval, I., Vanasse, A. Pageau, D. Dion, Y. 2015. Seeding rate and cultivar effects on yield, yield components and grain quality of spring spelt in eastern Canada. Canadian Journal of Plant Science, 95(5), 841-849

Erba, D., Hidalgo, A. Bresciani, J. Brandolini, A. 2011. Environmental and Genotypic Influences on Trace Element and Mineral Concentrations in Whole Meal Flour of Einkorn (*Triticum monococcum L. subsp. monococcum*), Journal of Cereal Science, 54 (2), 250-254.

Erdoğan, P., 2023. Farklı Ekim Sıklığı ve Azot Dozlarının Siyez Buğdayında (Triticum Monococcum L.) Verim ve Kalite Oğeleri Uzerine Etkisi (Master's thesis, Bursa Uludag University (Turkey)).

Eyüpoğlu, F., Kurucu, N. Talaz, S. 1995. Türkiye Topraklarının Bitkiye Yarayışlı Mikroelementler Bakımından Genel Durumu. Toprak Gübre Araştırma Ens.

Gabrovska, D., Fiedlerova, V. Holasova, M. Maskova, E. Smrcinov, H. Rysova, J. Winterova, R. Michalova, A. Hutar, M. 2002. The Nutritional Evaluation of Underutilized Cereals and Buckwheat., Food and Nutrition Bulletin, 23 (3 Suppl), 246-249.

Gee, GW., Bauder, JW. 1986. Particle-Size Analysis 1. *Methods of Soil Analysis: Part 1—Physical and Mineralogical Methods,* (methodsofsoilan1), 383-411.

Heun, M., Schäfer-Pregl, R. Klawan, D. Castagna, R. Accerbi, M. Borghi, B. Salamini, F. 1997b. Site of Einkorn Wheat Domestication Identified by DNA Fingerprinting, *Science*, 278 (5341), 1312-1314.

Hotz, C., Brown, KH. 2004. Assessment of the Risk of Zinc Deficiency in Populations and Options for Its Control. Food Nutr Bull 25:94–204

İbrikçi, H., Gülüt, K. Güzel, N. Büyük, G. 1994. Gübrelemede Bitki Analiz Teknikleri, *ÇÜ Ziraat Fakültesi Genel Yayın* (95).

Kan, M., Küçükçongar, M. Keser, M. Morgounov, A. Muminjanov, H. Özdemir, F. Qualset, C. 2015. Wheat Landraces in Farmers' Fields in Turkey National Survey, Collection and Conservation. Food and Agriculture Organization of the United Nations Ankara 1-157.

Kirchev, H., Semkova, N. 2016. Investigation on some morphological and biological characteristics of einkorn wheat (T.monococcum L.) depending on nitrogen fertilization. Journal of Agricultural, Food and Environmental Sciences, JAFES, 69, 69- 74.

Lev-Yadun, S., Gopher, A. Abbo, S. 2000. The Cradle of Agriculture, *Science*, 288 (5471), 1602-1603.

Lindsay, WL., Norvell, WA. 1978. Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper 1, *Soil Science Society of America Journal*, 42 (3), 421-428.

Loje, H., Moller, B. Laustsen, A. Hansen, A. 2003. Chemical Composition, Functional Properties and Sensory Profiling of Einkorn (*Triticum monococcum* L.), Journal of Cereal Science, 37 (2), 231-240.

McLeon, E., Cogswell, M. Egli, I. Wojdyla, D. Benoist, B. 2009. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. Pub Health Nutr 12:444–454

Nesbit, M., Samuel, L. 1998. Wheat Domestication, Archeobotanical Evidence. Science, 279, 14-33.

Nesbitt, M., Samuel, D. 1996. From Staple Crop to Extinction? The Archaeology and History of the Hulled Wheats. In: Padulosi S, Hammer K, Heller J (Eds) Hulled wheats. Proceedings of the First International Workshop on Hulled Wheats, 21-22 July 1995, Castelvecchio Pascoli Italy. IPGRI Rome pp 41-100.

Ohwaki, Y., Kraokaw, S. Chotechuen, S. Egawa, Y. Sugahara, K. 1997. Differences in Responses to Iron Deficiency Among Various Cultivars of Mungbean (Vigna radiata (L.) Wilczek), *Plant and Soil*, 192 (1), 107-114.

Olsen, SR., Sommers, LE. 1982. Sodyum Bikarbonatta Çözünen Fosfor. *Toprak Analiz Yöntemleri, kısım , 2* , 421-422.

Ozkan, H., Brandolini, A. Torun, A. Altintas, S. Eker, S. Kilian, B. Braun, H. Salamini, F. Cakmak, I. 2007. Natural Variation and Identification of Microelements Content in Seeds of Einkorn Wheat (*Triticum monococcum*), In: WheatProduction in Stressed Environments, Eds: Springer, p. 455-462.

Richards, LA. 1954. Diagnosis and Improvement of Saline and Alkaline Soils. U.S. Dep. Agr. Handbook, Washington.

Sillanpaa, M., 1982. Micronutrients and the Nutrient Status of Soils: a Global Study, 48, Food & Agriculture Org., p.

Suchowilska, E., Wiwart, M. Kandler, W. Krska, R. 2012. A Comparison of Macro and Microelement Concentrations in the Whole Grain of Four Triticum Species, Plant, Soil and Environment, 58 (3), 141-147.

Thomas, GW., 1982. Exchangeable Cations, Pgs 159-165 In: Methods of Soil Analysis. Part II (page A. L Miller. R. H., and Keeney. D. R., eds,). 2nd Edition.America Society of Agronomy and Soil Science of America. Madison. Wisconsin, USA.

Walkley, A., Black, IA. 1934. An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Science*, *37*(1), 29-38.

Welch, RM., Graham, RD. 1999. A new Paradigm for World Agriculture: Meeting Human Needs-Productive, Sustainable Nutritious. Field Crop Research 60:1-10

WHO, 2002. World Health Organization, The World Health Report, Geneva.

Zencirci, N., Karakaş, FP. Ordu, B. 2021. Macro-Micro Element Variation in Traditionally Grown Einkorn (Triticum monococcum L. subsp. monococcum) and Emmer Wheat (Triticum dicoccon Schrank). International Journal of Secondary Metabolite, 8(3), 227-245.

Zengin, G., 2015. Bazı İlkel Buğdaylarda Kalite Parametrelerinin Belirlenmesi Üzerine bir Araştırma, Selçuk Üniversitesi Fen Bilimleri Enstitüsü, Gıda Mühendisliği Ana Bilim Dalı Yüksek Lisans Tezi, 89 sy.

Zhao, F., Su, Y. Dunham, S. Rakszegi, M. Bedo, Z. McGrath, S. Shewry, P. 2009. Variation in Mineral Micronutrient Concentrations in Grain of Wheat Lines of Diverse Origin, Journal of Cereal Science, 49 (2), 290-295.

ROOT NODULE FORMATION ABILITY OF LEGUMES IN ANNUAL MIXTURES

Viliana Vasileva[1], Yalcin Kaya[2]*

[1]*Maize Research Institute, Knezha 5835, Agricultural Academy, Bulgaria*
[2]*Trakya University, Department of Genetic and Bioengineering, Edirne, Turkey*

Corresponding author e-mail: viliana.vasileva@gmail.com

ABSTRACT

The nodulation potential of legume components in annual grass-legume forage mixtures was investigated. Pea and vetch sown alone (100%), in double mixtures with oat in the legume:grass ratio (70:30%), and in triple mixtures in the legume:legume:grass ratio (35:35:30%) were tested in a pot trial under semi-controlled conditions. Spring forage pea variety Crystal and spring vetch variety Obrazets 666 were used. Based on plant morphometry - root mass, nodule characteristics, some biometric indicators of nodule formation were calculated. Pea plants (100%) showed greater specific nodule-forming ability compared to vetch (100%). The same tendency was found for the mixtures with oat (70:30%). In the triple mixtures (pea-vetch-oat - 35:35:30%), the specific nodule-forming ability of both leguminous crops decreased, weaker for pea (5.22%) versus vetch (13.66%).

Keywords: pea, vetch, nodulation, mixtures

INTRODUCTION

The permanent climate changes occurred in recent decades posed a serious risk to agricultural crops, reducing both their productivity and the quality of the production (Aranjueloa et al., 2014). Climate change projections in Europe predict an increase in temperatures, a decrease in rainfall and a dramatic increase in the frequency of drought years. Agriculture and biodiversity are most vulnerable to the effects of climate change. The increasing frequency and duration of drought periods require adaptation of crops to the changed conditions, and greater efficiency of resource use (Lionello and Scarascia, 2018; Grillakis, 2019).

Growing of crops in mixtures is considered as a one of the ways to use resources more efficiently. Mixed cropping is more efficient than monocropping in terms of using environmental resources for plant growth and development (Sebastia et al., 2004; Mahapatra, 2011). They are longer lasting, more resilient and better able to withstand adverse conditions (Porqueddu et al., 2003; Peyraud et al., 2009). Each species in the mixture contributes to a different degree to increase productivity (Vasilev et al., 2005;

Albayrak et al., 2011; Vasileva and Vasilev, 2012a,b). Many studies have proven the higher productivity of mixtures compared to single crops (Turk, 2000; Odhiambo and Bomke, 2001; Glacomini et al., 2003; Elessesser, 2004; Vasilev, 2004; Malhi et al., 2004; Shiferaw et al., 2004; Shisaya, 2005).

Legumes are a desirable component in mixtures due to their nitrogen-fixing ability. Mixtures with legume component (-s) included in their composition are more productive and resistant. An advantage of grass-legume mixtures is the possibility of supplying the grass component with nitrogen from the legume (-s) through symbiotic nitrogen fixation (Nyfeler et al., 2005, 2006; Peeters et al., 2006; Pozdisek et al., 2011). The nitrogen obtained through this process is one of the main elements of sustainable agriculture, so the mixed cultivation of perennial grasses and leguminous species also has an important ecological aspect (Dita et al., 2006; Luscher et al., 2014; Kusvuran et al., 2014). In the case of mixed grass-legume crops, a part of the nitrogen is transferred from the legume to the corresponding grass component in the mixture. Although most of the fixed nitrogen is available to the leguminous crop, some is released into the soil and becomes available to the grass crop. Competition for soil nitrogen in mixed systems can have a beneficial effect on stimulating nitrogen-fixing processes.

The ability to supply the grass components in the mixture with nitrogen from the legume through symbiotic nitrogen fixation reduces or eliminates the need to use nitrogen fertilizers, thus helps to improve nitrogen nutrition of plants (Gil and Fick, 2001). In mixtures, the amount of nitrogen decreases, legumes receive more nitrogen from the air and are less competitive with grasses for soil nitrogen. On the other hand, at appropriate ratios with grass components, legumes are stimulated to fix more nitrogen (Sesitisch et al., 2002). After decomposition of nodule biomass by legumes, additional nitrogen becomes available to grasses (Li et al., 2015). After ploughing a red clover/grass ryegrass mixture mowed for silage, Ledgard (1991) found a residual effect of 63.0 kg N/ha for the following crop (wheat). The mowed and chopped biomass and left on the ground during the fallow period provides 80-160 N/ha of available nitrogen for the next crop after the grass-legume mixtures, and 160-260 N/ha after single legume crops.

Studies on the nodulating status of leguminous forage crops would be a contribution to the effective use of their symbiotic nitrogen-fixing potential to increase forage productivity.

In the present study, we investigated the potential for nodule formation of legumes in annual grass-legume forage mixtures - pea-oat (70:30%), vetch-oat (70:30%) and pea-vetch-oat (35:35:30%).

MATERIAL AND METHOD

A pot trial was performed with the next variants of the study: pea (100%), vetch (100%), pea-oat (70:30%), vetch-oat (70:30%), pea-vetch-oat (35:35:30%), repetition – fourfold. Pots with a volume of 5 l were used. Spring forage pea variety Crystal and spring vetch variety Obrazets 666 were used. Under laboratory conditions, after washing the root system of leguminous crops, the following parameters were determined: on the root mass: root mass length (cm), specific root length (length of the root mass/fresh weight of the root mass) (cm/g), fresh top mass weight (g/plant), weight of dry root mass (g/plant) (drying 60 ^{o}C); nodulation - the number of nodules on the main and lateral roots of the plants was counted by counting and recalculating to the number of nodules per plant; nodule weight (g/plant), specific nodulating ability(g nodules/g root mass), rate of nodule formation according to the formula of Nif Tal Project of the University of Hawaii (1979): Rate of nodule formation =(aX10)+(bx5)+(cx1)+(dx0)/total number of plants,

where a - plants with nodules located along the main root; b - plants with tubers located on the lateral roots, but closer to the main root; c - plants whose noduless are scattered along the roots; d - plants without nodules; of top mass: height (cm), fresh top mass weight (g/plant), dry top mass weight (g/plant) (drying 60 ^{o}C), top mass/root mass ratio (length), top mass/root mass ratio (weight). Pearson's simple linear correlation coefficient (r) was calculated and correlation dependences between the studied indicators were found. Minimum, maximum values, STDEV, CV% were defined.

RESULTS AND DISCUSSION

Both pea and vetch are high productive annual legume crops (Kosev et al., 2019; Vasileva et al., 2020a, 2020b). Data were analyzed in relation to the habit of the plants, and their ability to form nodules in the beginning of flowering stage was investigated. Based on the morphometry of the plants – top mass, root mass, nodule parameters, some biometric indicators of nodule formation were calculated.

In terms of root mass, the length varied from 8.2 to 11.4 cm (Table 1). The weight of fresh root mass in the double mixtures decreased slightly for pea plants and by 8.8% for those of vetch. In the triple mixtures, the reductions were significant for both legume crops and ranged from 19.61 to 20.18%.

Table 1. Root mass of pea and vetch in annual mixtures

Variants	Root mass			
	length	SRL	fresh weight	dry weight
	cm	cm/g	g/plant	g/plant
Pea (100%)	11.4	4.286	2.66	0.66
Vetch (100%)	10.2	3.377	3.02	0.79
Pea-oat (70:30%)	11.1	4.007	2.77	0.75
Vetch-oat (70:30%)	9.3	4.408	2.11	0.69
Pea-vetch-oat (35:35:30%)	9.1	3.776	2.41	0.66
Pea-**vetch**-oat (35:35:30%)	8.2	2.724	3.01	3.02
Max	11.4	4.408	3.02	3.02
Min	8.2	2.724	2.11	0.66
STDEV	1.24	0.629	0.35	0.94

Bolding refers to the respective crop in the mixture

The weight of the fresh root mass in the double mixtures decreased to 30.1% in vetch and remained almost unchanged in pea. However, an opposite trend was observed in the triple mixtures, where the weight of fresh root mass in pea decreased by 9.40% and did not change in vetch.

In terms of nodule formation, pea plants (100%) (16.1 nodules/plant) had the highest nodule formation capacity (Table 2), followed by vetch (100%) (14.5 nodules/plant). In mixtures, the tendency for greater nodule -forming ability of pea compared to vetch is maintained, as it is determined by the ratio with the grass crop. In the triple mixtures (pea-vetch oat - 35:35:30%), the nodule formation of both pea and vetch plants decreased by 5.22% and 13.66%, respectively. The data for specific nodulating ability were similar.

Table 2. Parameters of nodule biomass of pea and vetch in annual mixtures

Variants	Nodules		
	number/plant	weigth	SNA
		g/plant	cm/g
Pea (100%)	16.1	0.3365	0.1265
Vetch (100%)	14.5	0.3031	0.1003
Pea-oat (70:30%)	15.0	0.3135	0.1132
Vetch-oat (70:30%)	14.0	0.2926	0.1387
Pea-vetch-oat (35:35:30%)	13.9	0.2905	0.1205
Pea-**vetch**-oat (35:35:30%)	13.7	0.2863	0.0951
Max	16.1	0.3365	0.1387
Min	13.7	0.2863	0.0951
STDEV	0.90	0.0188	0.0163

Bolding refers to the respective crop in the mixture

According to the level of nodule formation, legumes are ranked as follows (Figure 1): for pea - pea (100%) (62), pea-oat (70:30%) (58), pea-vetch-oat (35:35: 30%) (52); for vetch - vetch (100%) (60), vetch-oat (70:30%) (50), pea-vetch-oat (35:35:30%) (49).

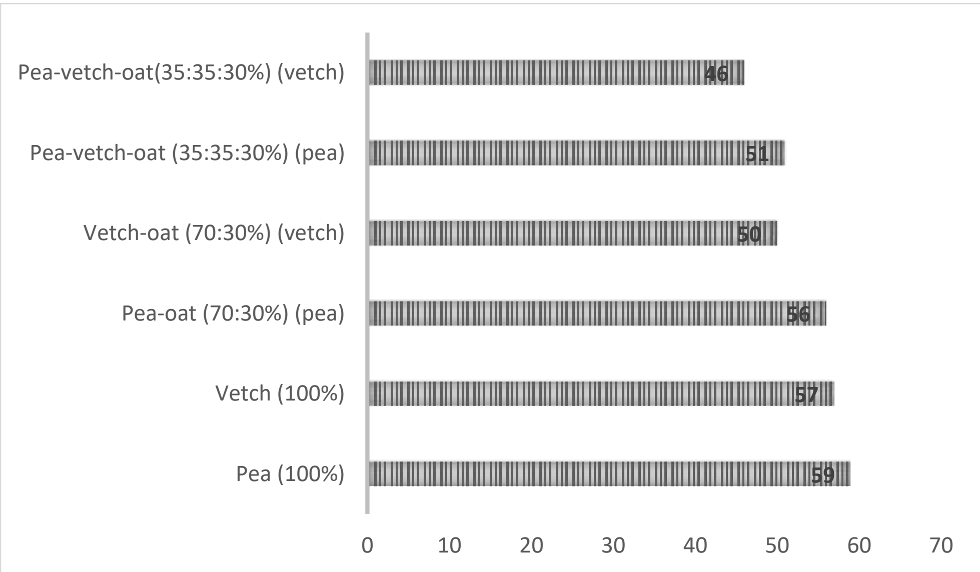


Figure 1. Nodule rate of pea and vetch in annual mixtures

Top mass height ranged from 48.6 to 55.9 cm (Table 3). In the triple mixtures (pea-vetch-oat - 35:35:30%) it decreased, respectively for pea up to 9.84% and insignificantly for vetchs (up to 3.57%).

Table 3. Top mass of pea and vetch in annual mixtures

Variants	Top mass		
	heigth	fresh weigth	dry weigth
	cm	g/plant	g/plant
Pea (100%)	55.9	9.30	2.34
Vetch (100%)	50.4	8.00	2.14
Pea-oat (70:30%)	51.2	8.99	2.31
Vetch-oat (70:30%)	49.8	7.68	2.02
Pea-vetch-oat (35:35:30%)	50.4	9.01	2.15
Pea-**vetch**-oat (35:35:30%)	48.6	7.56	1.99
Max	55.9	9.30	2.34
Min	48.6	7.56	1.99
STDEV	2.53	0.76	0.14

Bolding refers to the respective crop in the mixture

Some ratios between top mass and root mass (height, length, fresh and dry weight) of the plants were also determined, which ratios follow the trend established for the indicators themselves (Table 4).

Table 4. Top mass/root mass ratios of pea and vetch in annual mixtures

Variants	Top mass/Root mass		
	height/length	fresh weight	dry weight
Pea (100%)	4.904	3.496	3.545
Vetch (100%)	4.941	2.649	2.709
Pea-oat (70:30%)	4.613	3.245	3.080
Vetch-oat (70:30%)	5.355	3.640	2.928
Pea-vetch-oat (35:35:30%)	5.538	3.739	3.258
Pea-**vetch**-oat (35:35:30%)	5.927	2.512	0.659
Max	5.927	3.739	3.545
Min	4.613	2.512	0.659
STDEV	0.483	0.520	1.038

Bolding refers to the respective crop in the mixture

Some correlation dependences were also established between the studied indicators through the Pearson linear correlation coefficient (r) (Table 5).

A strong positive correlation was found between length of root mass and number and weight of nodules (r=0.9225), as well as between specific length of roots and specific nodule ability (r=0.9194).

A significant positive correlation was found between the height of the top mass and the specific length of the roots (r=0.5730), between the weight of the fresh top mass and the length of the root mass (r=0.7011), between the specific length of the roots and the number and weight of nodules (r=0.5157).

A moderate positive correlation was found between the height of the top mass and the specific nodulating ability (r=0.3901).

A weak positive correlation was found between specific nodulating ability and: fresh weight of top mass (r=0.2900), dry weight of top mass (r=0.1789), length of root mass (r=0.2560), number and weight of nodules (r=0.2459).

Table 5. Correlation coefficients for the parameters studied

	TMH	FTMW	DTMW	RML	SRL	FRMW	DRMW	NNo	NW	SNA
FTMW	0.7543									
DTMW	-0.6925	0.9024								
RML	0.8110	0.7011	0.9242							
SRL	0.5730	0.4941	0.4958	0.5986						
FRMW	-0.0502	-0.0961	0.1147	0.0798	-0.7473					
DRMW	-0.4934	-0.5728	-0.5732	-0.6565	-0.8287	0.5119				
NNo	0.9498	0.6976	0.8866	0.9225	0.5157	0.1248	-0.4574			
NW	-0.7511	0.6976	0.8866	0.9225	0.5157	0.1248	-0.4574	1.0000		
SNA	0.3901	0.2900	0.1789	0.2560	0.9194	-0.9272	-0.6505	0.2459	0.2459	
NR	0.7845	0.6540	0.8627	0.9487	0.4903	0.1668	-0.6945	0.8679	0.8679	0.1512

TMH, top mass height (cm); FTMW, fresh top mass weight (g/plant); DTMW, dry top mass weigth (g/plant); RML, root mass length (cm); SRL, specific root length (cm/g); FRMW, fresh root mass weight (g/plant); DRMW, dry root mass weight (g/plant); NNo, nodule number; NW, nodule weight (g/plant); SNA, specific nodulating ability (g nodules/g root mass), NR, nodule rate

CONCLUSIONS

Data were analysed in relation to the habit of pea and vetch plants, and their ability to form nodules in pea-oat (70:30), vetch-oat (70:30%) and pea-vet-oat (35 :35:30%) mixtures. Based on plant morphometry – top mass, root mass, nodule parameters, some biometric indicators of nodulation were calculated. Pea plants (100%) (16.1 nodules/plant) were found to have the highest nodulating ability, followed by vetch (100%) (14.5 nodules/plant). In mixtures, the tendency for greater nodule-forming ability of pea compared to vetch was maintained, as it is determined by the ratio with the grass crop. In the triple mixtures (pea-vetch-oat - 35:35:30%), the nodule formation of both pea and vetch plants decreased by 5.22% and 13.66%, respectively.

A strong positive correlation was found between length of root mass and number and weight of nodules (r=0.9225), as well as between specific length of roots and specific nodule ability (r=0.9194).

A weak positive correlation was found between specific nodulating ability and fresh weight of top mass (r=0.2900).

REFERENCES

Albayrak, S., Turk, M., Yuksel, O., Yilmaz, M. 2011. Forage yield and the quality of perennial legume-grass mixtures under rainfed conditions. Not. Bot. Hort. Agrobot. Cluj. 39:114-118.

Aranjueloa, I., Arrese-Igor, C., Moleroc, G. 2014. Nodule performance within a changing environmental context. Journal of Plant Physiology, 171, 1076–1090.

Dita, M.A. Rispail, N., Prats, E., Rubiales, D., Singh, K.B. 2006. Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica 147: 1-24.

Elessesser, M. 2004. Optimum management intensity of legume and grass based grassland swards, pp. 510-512. Proc. 20th General Meeting of European Grassland Federation, Switzerland.

Gil, J. L., Fick, W.H. 2001. Soil nitrogen mineralization in mixtures of eastern gamagrass with alfalfa and red clover. Agron. J. 93, 902-910.

Glacomini, S., Aita, J.C., Vendruscolo, E.R.O., Cubilla, M., Nicoloso, R.S., Mreries M. R. 2003. Dry matter, C/N ratio and nitrogen, phosphorus and potassium accumulation in mixed soil cover crops in Southern Brazil [Portuguese]. Rev. Bras. Ciene. Solo. (27): 325-334.

Grillakis M.G. 2019. Increase in severe and extreme soil moisture droughts for Europe under climate change. Science of the Total Environment, 660, 1245-1255.

Kosev V., Vasileva V., Kaya Y. (2019). Assessment of Initial Material of Vetches in Productive and Adaptive Ability. International Journal of Innovative Approaches in Agricultural Research, **ISSN**: 2602-4772 (online), 3(1), 96-111. doi: 10.29329/ijiaar.2019.188.10

Kusvuran, A., Ralice, Y., Saglamtimur, T. 2014. **Determining the Biomass Production Capacities of Certain Forage Grasses** and **Legumes and their Mixtures under**

Mediterranean Regional Conditions. Acta Advances in Agricultural Sciences, (2): 13-24.

Ledgard, S.F. 1991. Transfer of fixed nitrogen from white clover to associated grasses in swards grazed by dairy cows estimated using ^{15}N methods. Plant and Soil, 131, 215-223.

Li, X., Sorensen, P., Li, F., Petersen, S.O., Olesen, J.E. 2015. Quantifying biological nitrogen fixation of different catch crops, and residual effects of roots and tops on nitrogen uptake in barley using in-situ 15N labelling. Plant and Soil 395:273-287

Lionello P., Scarascia L. 2018. The relation between climate change in the Mediterranean region and global warming. Regional Environmental Change, 18 (5), 1481-1493.

Luscher, A., I. Mueller-Harve, I. F. Soussana, R.M. Rees, J.L. Peyraud 2014. Potential of legume-based grassland-livestock systems in Europe: a review. Grass and Forage Science 69: 206-228.

Malhi, S.S., K.S. Gill, D.H. Mcartney, R. Malmgren 2004. Fertilizer management of forage crops in the Canadian Great Plains. Crop Sci. 1: 237-271.

Nif Tal 1979. Legume Inoculation Trials Procedures. Nif Tal Project, University of Hawaii, Honolulu, Hawaii, USA.

Nyfeler, D., O. Huguenin-Elie, E. Frossard, A. Luscher 2005. Symbiotic nitrogen fixation during the first year of the COST 852 common experiment in Switzerland. Workshop on Sward dynamics, N-flows and forage utilisation in legume-based systems, November 10-12, 2005 Grado (Italy) COST Action 852.

Nyfeler, D., O. Huguenin-Elie, E. Frossard, A. Luscher 2006. Regulation of symbiotic nitrogen fixation in grass-clover mixtures. In: Lloveras J., A. Gonzales-Rodríguez, O. Vazquez-Yañez, J. Piñeiro, O. Santamaría, L. Olea and M. J. Poblaciones (eds.). Grassland Science in Europe, 11, 246-248.

Odhiambo, J.J.O., A.A. Bombe 2001. Grass and legume cover crop effects on dry matter and nitrogen accumulation. Agron. J. 93: 299-307.

Peeters, A., Parente, G., Gall, A. 2006. Temperate legumes: key –species for sustainable temperate mixtures. In: Sustainable Grassland Productivity. Lloveras J., Gonzales-Rodríguez A., Vazquez-Yañez O., Piñeiro J., Santamaría O., Olea L. and Poblaciones M. J. (Eds.). Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, 3-6 April, 2006. Grassland Science in Europe, (11): 205-220.

Peyraud, J. L., A. Le Gall, Lüscher A. 2009. Potential food production from forage legume-based-systems in Europe: An overview. Irish J. Agric. Food Res. 48: 115–135.

Porqueddu, C., G. Parente, M. Elsaesser 2003. Potential of grasslands. In: A. Kirilov, N. Todorov and I. Katerov (eds.). Grassland Science in Europe, (8): 11-20.

Pozdisek, J., Henriksen, B., Ponizil, A., Loes, A. K. 2011. Utilizing legume-cereal intercropping for increasing self-suffiency on organic farms in feed for monogastric animal. Agron. Res. 9: 343-356.

Sebastia, M. T., A. Luscher, J. Connolly, R. Collins, I. Delgado, A. De Viegher, P. Evans, M. Fothergill, B. Frankow-Lindberg, A. Helgadottir, C. Illiadis, M. Jorgensen, Z. Kadziuliene, F. Nissines, D. Nyfeler, C. Porqueddu 2004. Higher yield and fewer weeds in grass/legume mixtures than in monocultures- 12 sites of COST action 852. Grassland Science in Europe, 9, 483-485.

Shiferaw, B., M.C.S. Bantilan, R. Serraj 2004. Symbiotic nitrogen fixation; prospects for enhanced application in tropical agriculture. Oxford and IBH publishing Co. (Pvt.) Ltd., New Delhi, India.

Turk M.A. 2000. Productivity of barley and common vetch under different cropping systems in a Mediterranean type environment. Crop Res. Hisar. 19: 175-183.

Vasilev, E., V. Vasileva, Tz. Mihovsky, G. Goranova 2005. Assessment of legume based mixture swards constrained by the environmental conditions in Central North Bulgaria- COST Action 852. Sward dynamics, N-flows and Forage Utilisation in Legume-Based Systems. Wachendorf M., Helgadottir A., Parente G. (eds.). Proceedings of the 2nd COST 852 workshop held in Grado, Italy 10-12 November 2005, 177-180.

Vasileva V., Kosev V., Kaya Ya. (2020a). Evaluation of forage pea lines in regard to earliness and grain yield. PROCEEDINGS OF II. INTERNATIONAL AGRICULTURAL, BIOLOGICAL & LIFE SCIENCE CONFERENCE, September 1-3, 2020, Edirne, Turkey, ISBN #:978-975-374-279-5, 1145-1150.

Vasileva V., Kosev, V., Kaya, Y. 2020b. Study of initial material of vetch. **The International Journal of Innovative Approaches in Agricultural Research (IJIAAR), 4 (2): 231-241.**

Vasileva, V., Vasilev, E. (2012a). Dry mass yield from some pasture mixtures with subterranean clover (*Trifolium subterraneum* L.). Journal of Mountain Agriculture on the Balkans, 15 (5): 1024-1033.

Vasileva, V., Vasilev, E. (2012b). Study on Productivity of some Legume Crops in Pure Cultivation and Mixtures. Agriculturae Conspectus Scientificus, 77 (2): 91-94.

ESSENTIAL OIL OF ERINACEA ANTHYLLIS LINK (FABACEAE): COMPOSITION AND ANTIBACTERIAL EFFECTS

Sabrina Bicha[1],Souhir Boureghida[1], Faten Abada[1], Nabila Slougui[1 2], Hamza Fadel[1], Hicham Hazmoune[1],*

[1] Research Unit: Valorization of Natural resources, Bioactive molecules and Analyses Physicochemical and Biological (VARENBIOMOL), Department of Chemistry, Faculty of Science, University Mentouri Constantine1, 25000 Constantine, Algeria.
[2]National polytechnic school of Constantine

** Corresponding author, Bicha Sabrina tel/: 00213540898581, email: bichasabrina2016@gmail.com*

ABSTRACT

Erinacea species is used in traditional medicine to treat rheumatic diseases.The aim of this study was to determine for the first time the chemical composition and antibacterial activity of essential oil from the fresh aerial parts of *Erinacia anthyllis* belonging to the Fabaceae family. The steam distilled oil was analyzed using gas chromatography techniques (GC-FID and GC-MS) using two different stationary phase columns (polar and non polar). Furthermore, antibacterial activity against various gram-positive and negative bacteria was determined by disk diffusion and microdilution. A total of 44 volatile substances were identified, accounting for about 98.22% of essential oils. The main components were phytol (9.26%), tricosane (8.62%). In addition, essential oils exhibit effect on all bacterial strains. The obtained inhibition zone ranged from 7mm to 21mm with the highest inhibition zone recorded for*Bacillus Sp* (21mm).*Erinacea anthyllis* essential oil has good antimicrobial activity against all tested pathogenic bacteria and may be used as a natural antimicrobial agent in the treatment of many infectious diseases.

Keywords: *Erinacea anthyllis;*antibacterial*;* essential oil; Batna; Algeria

INTRODUCTION

The Fabaceae family, known as Leguminosae, is a large and diverse family of flowering plants that includes over19000 species. It is one of the most economically important plant families, as many species are used for food, fodder, fuel and medicinal purposes.[1-2]
Several species within the Fabaceae family possess antibacterial proparties against a range of pathogenic bacteria, including *Escherichia coli*, *Salmonella*, *Staphylococcus aureus* and *Streptococcus* pyogenes.[3-7] The genus *Erinacea* belongs to the legume family , the tribe Genisteae, while is represented by a single species called *Erinacea anthyllis* Link or *Erinacea*

pungens Boiss.[8] *Erinacea anthyllis*, also known as blue broom, hedgehog plant, or rushy kidney vetch, is a species of flowering plant. It is a dwarf, spiny shrub that grows under 30cm tall and has erect branches terminating in sharp spines. The leaves are inconspicuous and the flowers are blue and purple. It is native to Spain and Algeria. Moreover, it is a rare and choice spiny legume.[9] *E anthyllis* is used in traditional medicine in Algeria to treat rheumatic diseases and as a honey source in the siroua region of Morocco.[10] *Erinacea anthyllis* contains a variety of secondary metabolites including polyphenols, flavonoids and steroids. Two new prenylated isoflavonoids named ErinasoneA and ErinasoneB were isolated from this plant, along with 19 known secondary metabolites. These two isoprenylated isoflavonoids have been found to have antioxidant and antibacterial activities.[11] There is no information about *E.anthyllis* essential oil in the provided search results. The retrieval results mainly provide information on taxonomy, morphology, habitat, and chemical composition of this plant. Therefore, in this study, the chemical constituents of essential oils extracted from the aerial parts of *E. anthyllis* were examined. In addition, the antibacterial ability against four common pathogens was also evaluated.

MATERIALS AND METHODS

Plant collection and identification

Stems, leaves, flowers of *Erinacea anthyllis* Link. were collected during the flowering stage in May 2022 from Batna, eastern of Algeria. Plant materials were authentically identified by Prof. Khellaf REBBAS, Department of Biological Sciences, University of M'sila. A voucher specimen (Number N°VAREAT5/22) was deposited at the Herbarium of VARENBIOMOL unit,Constantine, Algeria.

Extraction of essential oil

The fresh aerial part of *Erinacea anthyllis* (150 g) was subjected to steam distillation in a Kaiser Lang apparatus for three hours. The resulting oil (750 mg) was collected and dried over anhydrous sodium sulphate and kept at 4 °C until analysis. Oil yield is calculated based on plant weight.

GC-FID and GC-MS analysis

GC-FID essential oil components were analysed using a SCHIMADZU GC-2010 chromatograph equipped with Rxi-5ms capillary column (30 m * 0.25 mm, film thickness 0.25 µm). Helium was the carrier gas, at a flow rate of 1.44 mL/min. The oven temperature was maintained at 45°C for 10 min and then increased to 180°C at a rate of 3°C/min and maintained at 180°C for 5 min, then to 280°C at a rate of 5°C/min and maintained at 280°C for 5 min and finaly to 330°C at a rate of 10°C/min for 2 min. Injector and detector (FID) temperatures were set at 330 °C. Diluted samples (in dichloromethane) of 1 µL were injected in the split/splitless (30:1 split) mode GC-MS analysis was performed using GCMS-QP2010. The Mass selective detector was equipped with a capillary column Rxi-5ms capillary column (30 m * 0.25 mm, film thickness 0.25 µm). Helium was the carrier gas, at a flow rate of 1.44 mL/min. The oven temperature was maintained at 45°C for 10 min and then increased to 180°C at a rate of 3°C/min and maintained at 180°C for 5 min, then to 280°C at a rate of 5°C/min and maintained at 280°C for 5 min and finally to 330°C at a rate of 10°C/min for 2 min. For GC-MS detection, an electron ionization system with ionization energy of 70 eV, was used. Injector temperature was: 330°C. Diluted sample (in dichloromethane) of 1 μL were injected in the split/splitless (30:1 split) mode. Identification of oil components issued from capillary column was accomplished based on comparison of their retention index to those of literature and also comparison of their mass fragmentation patterns with those of databases. [12]

Antimicrobial assay

E.antyllis oil has been evaluated against four common pathogens, *Bacillus, Staphylococcus aureus*(ATCC25923), *Escherichia coli*(ATCC25922), *and Pseudomonas aeruginosa* (ATCC27853). To study antibacterial activity, sterile Muller Hinton agar medium was prepared in petri dishes, and yeast starch agar was inoculated separately into the medium alone.aseptically prepare four wells (6 mm in diameter) and add 80 □L of each extract prepared in DMSO to the wells. Plates were incubated at 37°C for 24 hours before the zone of inhibition was measured. At the end of the incubation period, the zone of inhibition was measured. [13]

Minimum inhibitory concentration determinations (MIC)

The minimal inhibitory concentration (MIC) , is defined as the lowest dose of antibiotic that inhibits observable bacterial growth after 24 hours of incubation. In this regard, the essential oil of *E. anthyllis* was diluted decimally with dimethyl sulfoxide (DMSO) and 10 μl of each dilution was pipetted into a sterilize paper disc (ø6 mm) placed in a petri dish. Petri dishes containing previously inculated Mueller-Hinton agar containing the culture of the bacteria to be tested, a negative control is also included in the test. The possible activity of the solvent against the bacteria to be tested is checked using a paper disc soaked in DMSO. The dishes were then incubated at 37°C for 24 hours. [14]

RESULTS AND DISCUSSION

Chemical constituents of essential oil

The steam distillation of *E. anthyllis* aerial part yielded a yellow essential oil with perfumery odour (0.35%, w/*w*). Identification of compounds was carried out using Target, Wiley 8, FFNSC1.2, NIST11, and Adams data bases.[12] These identifications were confirmed by linear retention indices (RI). As showen in Table 1, we detected 44 compounds representing 98.22% of the total composition. The main compounds were phytol (9.26%), tricosane (8,62%), □-thujone (7.66%), Artemesia ketne (6.64%), □□Muurolene (5.74%), 2-hexadecanone 6,10,14 trimethyl (6.22%) and Hexacosane (5.56%). Phytol and tricosane are two chemical compounds that are foundin many essential oils,including some varieties of lavender, lemongrass, and peppermint.Moreover, Bthujone is another compound that is found in some Fabaceae essential oils,including wormwood and sage. [15]

Phytol is a diterpene alcohol that is believed to have anti-inflammatory and antioxidant properties, and it is often used in the fragrance industry.[16] Tricosane is a straight chain hydrocarbon that is commonly found in plant waxes and has been used in some skincare products. [17] B-thujone is a monoterpene that is found in a variety of plants including sage.It is known for its psychoactive properties and has been used in some traditional medicines. [18]

E.anthyllis essential oils contain a complex mixture of constituents and thus have various antimicrobial properties, the bulk of this effect appears to come from oxygenated terpenoids, especially phenolic terpenes, phenylpropanes, and alcohols.

Antimicrobial activity

The antimicrobial potential of *Erinacea anthyllis* essential oil was assessed by the zone of inhibition of bacterial growth. The results in Table 2 demonstrated that essential oils have an effect on all bacterial strains. The zones of inhibition varied from 7mm to 21mm with highest zone recorded with *Bacillus Sp* (21mm).MIC values against *Echerichia coli*, *Staphylococcus aureus*,*Pseudomonas aeruginosa* and *Bacillus sp* are 1/4, 1/8,1/4,1/4 respectively.

Half-dilution of essential oils (Table 2) reduced the growth density of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and Bacillus, but tested bacteria were resistant to 1/8 of the essential oil concentration, except *Staphylococcus aureus*. The concentrated oil exhibited high levels of activity against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Bacillus, with inhibition diameters of 15 mm, 15 mm, 10 mm, and 21 mm, respectively, indicating that these bacteria are very sensitive. E. anthyllis essential oils had very small CMI values (1/4 and 1/8) for the tested microorganisms. These data are important for treating infections caused by these bacteria: *Staphylococcus aureus* is the leading cause of infection because of its virulence and ability to acquire antimicrobial resistance, causing serious problems for hospitals and healthcare professionals worldwide problem.[19] *Erinacea antyllis* essential oil has good antibacterial activity and may be used as a natural antibacterial agent in the treatment of various infectious diseases caused by Gram-positive and Gram-negative bacteria.

CONCLUSION

For the first time, the chemical composition of *E.anthyllis* essential oil and antibacterial activity were investigated. A total of 44 compounds were identified, accounting for 98.22%of the total oil.Good antibacterial effect of *Erinacea* essential oil has been observed, indicating its high potential as a new source of antibacterial agent. These effects can be traced back to their chemical constituents, such as phytol, tricosane, □□thujone,and □-Muurolene.

ACKNOWLEDGEMENTS

The authors thank the DGRSDT-Algeria for providing a research grant (PRFU Project B00L01UN250120220011)

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest

REFERENCES

1. Ahmad F, Anwar F, Hira S. Review on medicinal importance of Fabaceae family. Pharmacologyonline.2016; 3:151-157.

2.Doudach S, Slougui N, Rebbas K, Benmkhebi L, Bensouici C, Mehmet N A, Akkal S, Bicha S. First report on phytoconstituents, lc-esi/ms profile and in vitro antioxidant activities of *lathyrus latifolius* growing in Algeria. IJNES. 2022; 16(3): 128-136.

3. Das S, Sharangi A B, Egbuna C, Jeevanandam J, Ezzat S M, Adetunji C O, Onyeike P C. Health benefits of isoflavones found exclusively of plants of the fabaceae family. Functional Foods and Nutraceuticals Bioactive Components. Formulations and Innovations. 2020 ; 473-508.

4. Ramadhani U P, Chandra B, Rivai H. Overview of phytochemistry and pharmacology of chickpeas (*Phaseolus vulgaris*). WJPPS.2020; *9*(9):442-61.

5. Saad A M, Sitohy M Z, Ahmed A I, Rabie N A, Amin S A, Aboelenin S M, El-Saadony M T. Biochemical and functional characterization of kidney bean protein alcalase-hydrolysates and their preservative action on stored chicken meat. Molecules.2021; *26*(15): 4690.

6. Mosallam F M, El-Sayyad G S, Fathy R M, El-Batal A I. Biomolecules-mediated synthesis of selenium nanoparticles using *Aspergillus oryzae* fermented *Lupin* extract and gamma radiation for hindering the growth of some multidrug-resistant bacteria and pathogenic fungi. Microbial pathogenesis. 2018 ; 122 *:* 108-116.

7. Porras G, Chassagne F, Lyles J T, Marquez L, Dettweiler M, Salam A M, Quave C L. Ethnobotany and the role of plant natural products in antibiotic drug discovery. Chemical reviews.2020 ; 121(6) :3495-3560.

8. Adanson M. Familles des Plantes. II partie. (Ed). Vincent, Paris, France ; 1763 ; 1-640.

9. Maire R. Flore de l'Afrique du Nord., (Ed). Le chevalier, Paris ;1952-1987 ; Vol. 16

10.Birouk A, Consultant national en biodiversité et ressources phytogénétiques.Projet FAO / TCP/ MOR/3201,Maroc. 2009 ;1-34.

11. Mouffouk S, Marcourt L, Benkhaled M, Boudiaf K, Wolfender J L, Haba H.Two new prenylated isoflavonoids from *Erinacea anthyllis* with antioxidant and antibacterial activities. NPC. 2017 ; 12(7) : 1065-1068.

12. Adams RP. Identification of essential oil components by gas chromatography/ quadrupolemass spectrometry, Allured Publ., Carol Stream, IL, USA; 2007.

13. Raho G B, Otsmane M, Sebaa F. Antimicrobial activity of essential oils of *Juniperus phoenicea* from North Western Algeria. JMB.2017; 1: 01-07.

14. Wiegand I, Hilpert K, Hancock R E. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature protocols. 2008; 3(2):163-175.

15. Stojanović G S, Lckovski J D, Đorđević A S, Petrović G M, Stepić K D, Palić I R, Stamenković J. G. The first report on chemical composition and antimicrobial activity of *Artemisia scoparia* Waldst et Kit. extracts. N PC, (2020);15(3)

16. Silva R O, Sousa F B M, Damasceno S R, Carvalho N S, Silva V G, Oliveira F R M, Medeiros J V R. Phytol, a diterpene alcohol, inhibits the inflammatory response by reducing cytokine production and oxidative stress. Fundamental & clinical pharmacology. 2014; 28(4): 455-464.

17. Perera M A D, Qin W, Yandeau-Nelson M, Fan L, Dixon P, Nikolau B J. Biological origins of normal-chain hydrocarbons: a pathway model based on cuticular wax analyses of maize silks. The Plant Journal.2010; 64(4): 618-632.

18. Mighri H, Hajlaoui H, Akrout A, Najjaa H, Neffati M. Antimicrobial and antioxidant activities of *Artemisia herba-alba* essential oil cultivated in Tunisian arid zone. Comptes Rendus Chimie. 2010; 13(3) : 380-386.

19 .Carvalho M J, Pimenta FC, Hayashida M, Gir E, Silva A M, Barbosa C P, Canini SRMS, Santiago S. Prevalence of methicillin-resistant and methicillin- susceptible *S.aureus* in the *salvia* of health professionals.Clinics.2009;4:295-302

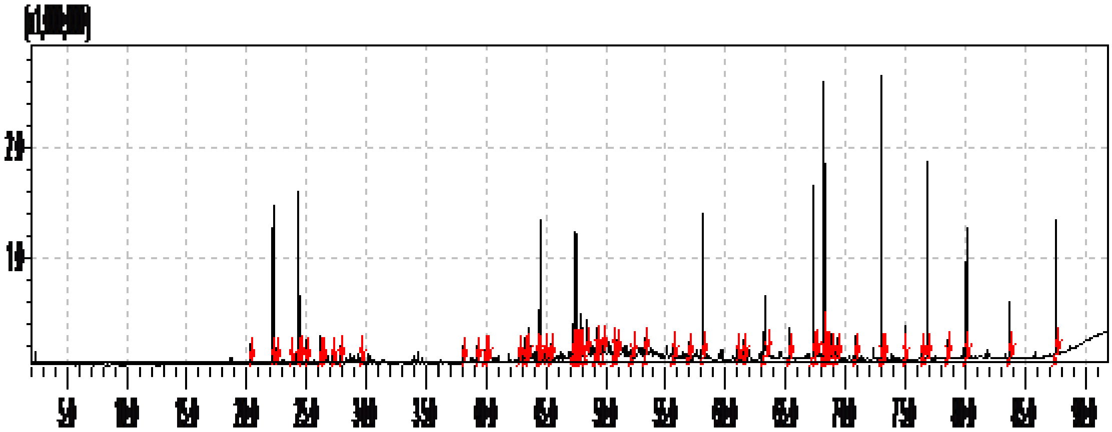


Figure 1:GC-FID chromatogram of *Erinacea* essential oil

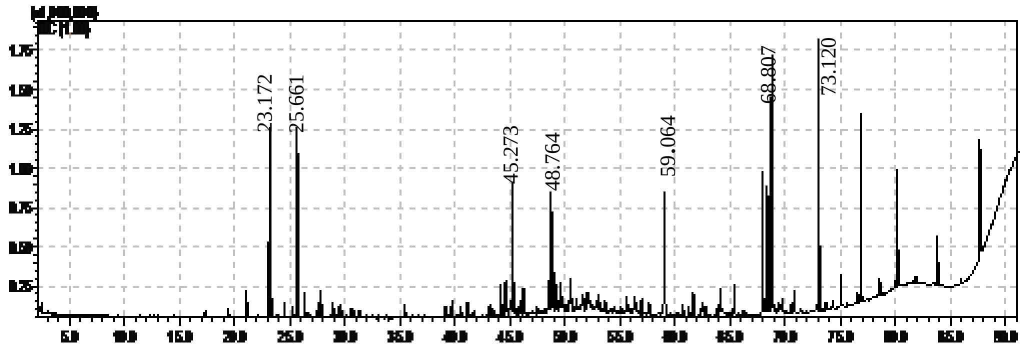


Figure 2: GC-MS chromatogram of *Erinacea* essential oil

Table 1. Chemical composition of *Erinacea anthyllis*. essential oils from Batna (Algeria)

Peak no.	Tr	RI	Components	%
01	21.102	1026	Eucalyptol	0.92
02	23.172	1056	Artemisia ketone	6.64
03	23.397	1065	Cis thujanol	0.64
04	24.515	1079	Artemisiaalcohol	0.48
05	25.661	1062	□ thujone	7.66
06	26.299	1125	□ thujone	0.88
07	27.532	1139	Trans-Pinocarveol	0.48
08	27.822	1141	Camphor	0.92
09	28.884	1164	Pinocarvone	0.48
10	29.525	1172	3-pinanone	0.48
11	39.142	1361	Nerolacetate	0.28
12	39.725	1374	□ -copaene	0.60
13	41.144	1402	Lauraldehyde	0.48

14	44.192	1477	□ Chamigrene	1.26
15	44.603	1487	E,□ Ionone	1.30
16	45.273	1500	□- Muurolene	5.74
17	45.431	1504	□-Himachalene	0.4
18	46.234	1523	□- Cadinene	1.0
19	48.517	1578	Spathulenol	1.24
20	48.764	1583	Caryophylleneoxide	5.10
21	49.110	1592	viridiflorol	1.98
22	49.220	1612	Cetane	0.9
23	49.602	1619	Cubenol (1,10-di-epi)	1.46
24	50.551	1630	Muurola-4,10(14)-dien-1-Beta-ol	1.12
25	51.580	1685	Cedranol (5-neo)	0.56
26	53.116	1700	heptadecane	0.56
27	55.653	1784	2.3-dimethoxy naphtalene	0.6
28	56.982	1800	octadecane	0.62
29	59.064	1854	2-hexadecanone,6,10,14-trimethyl	6.22
30	61.694	1900	Nonadecane	1.18
31	65.451	2000	Eicosane	0.96
32	67.974	2090	Cosyl alcohol	5.10
33	68.422	2100	Heneicosane	4.5
34	68.807	2106	phytol	9.26
35	69.820	2184	3,7,11,15-tetramethyl-2-octadecen-1-ol	0.48
36	70.915	2200	Docosane	0.6
37	73.120	2300	tricosane	8.62
38	75.90	2400	tetracosane	0.88
39	76.612	2549	1-tetracosanol	0.34
40	76.914	2600	Hexacosane	5.56
41	78.611	2700	Heptacosane	0.44
42	80.218	2800	Octacosane	3.60
43	83.864	3000	triacontane	1.90
44	87.071	3200	Docontane	3.8
Oil yield				**0.50**
Total identified				**98.22%**

Tr: Retention time obtained by chromatogram.
RI: Retention Index

Table 2. Antibacterial activity of *Erinacea anthyllis* essential oil

Bacterial strains	Inhibition zone (mm) with different essential oil concentrations				
	Pure oil 1/1	**Dilution 1/2**	**Dilution 1/4**	**Dilution 1/8**	**MIC**
Escherichia coli	15	7	7	0	1/4
Staphylococcus aureus	15	13	10	7	1/8
Pseudomonas aeruginosa	10	12	8	0	1/4
Bacillus sp	21	7	7	0	1/4

PROBABILISTIC HEALTH RISK ASSESSMENT OF FLUORIDE IN WATER OF NATURAL – ARTIFICIAL LENTIC HABITATS OF GÖKÇEADA ISLAND (TÜRKIYE)

Meltem Tekbaş Atay[1], Mediha Büyükgöze Dindar[2], Şirin Güner Onur[3], Cem Tokatlı[4]*

[1]*Trakya University, Faculty of Dentistry, Edirne / Türkiye*

[2]*Trakya University, Health Services Vocational School, Edirne / Türkiye*

[3]*Altınbaş University, Faculty of Dentistry, İstanbul / Türkiye*

[4]*Trakya University, İpsala Vocational School, Edirne / Türkiye*

**Corresponding author: meltemtekbasatay@trakya.edu.tr*

ABSTRACT

Located in the north of the Aegean Sea and at the entrance of Saros Gulf, Gökçeada is the largest island of Türkiye. The island is home to important freshwater resources and there are 2 dam lakes (Zeytinli and Uğurlu Dams), 3 ponds (Aydıncık, Şirinköy and Uğurlu Ponds) and a lagoon (Gökçeada lagoon). The aim of the current research was to assess the non-carcinogenic health risks of fluoride via daily human intake in water of natural – artificial lentic habitats of Gökçeada Island of Türkiye. 6 natural – artificial lentic habitats were selected on the island and water samples were collected during the dry – wet seasons of 2022 – 2023. The fluoride levels were measured by using spectrophotometric method and Estimated Daily Intake (EDI) and Hazard Quotient (HQ) of fluoride were calculated. As a result of this investigation, all the calculated HQ values both in dry and wet seasons in terms of all age groups were recorded as less than the critical limit of 1 with an annualy mean of 0.099 for infants, 0.703 for children, 0.496 for teenagers and 0.398 for adults.

Keywords: Gökçeada Island, Natural – Artificial Lentic Habitats, Health Risk Assessment

INTRODUCTION

Health risk assessment methods are being widely used all over the world in especially recent years. They are quite useful and effective tools in estimating carcinogenic and non-carcinogenic hazards, which may occur when people are exposed to certain toxicants (Ustaoğlu and Aydın, 2020; Tokatlı and Ustaoğlu, 2020; Tokatlı and Varol, 2021a; 2021b; Varol and Tokatlı, 2022; Tokatlı et al., 2022; 2023).

Gökçeada is the largest island in Türkiye with a surface area of 289.5 square kilometers and a coastline of 95 kilometers. Gökçeada, like all the islands in the Aegean Sea, has a volcanic ground and consists of rocks formed in the young geological period. The general geographical feature of the island is forest, maquis and olive groves and although, Gökçeada is generally suitable for all kinds of plant cultivation due to its fertile soil structure, olive groves are among the most important agricultural products of the island. In general, the continental climate is observed in the northern part of the island, and the characteristics of the Mediterranean climate are observed in the southern parts (Anonymous, 2022; http://www.gokceada.gov.tr/; https://www.gokceada.bel.tr/; https://www.kulturportali.gov.tr/).

The aim of the current research was to assess the non-carcinogenic health risks of fluoride via daily human intake in water of natural – artificial lentic habitats of Gökçeada Island of Türkiye.

MATERIALS AND METHODS

Water Collection

In this research, 6 natural – artificial lentic habitats (1 natural lake, 2 dam lakes and 3 artificial pons) located in the Gökçeada Island were selected. The coordinate information with the names of investigated freshwater ecosystems are given in Table 1. The topographic map of Gökçeada Island and selected locations are given in Figure 1. Water samples were collected in the summer season of 2022 (dry season) and the winter season of 2023 (wet season) with a telescopic water sampling device approximately 3 meters from the shore into the pre – cleaned polyethylene bottles.

Table 1. Coordinate information of selected stagnant water bodies

Name of Lentic	Station Code	GPS-North	GPS-East
Gökceada Lagoon	L1	40.133	25.956
Zeytinli Reservoir	R1	40.176	25.863
Uğurlu Reservoir	R2	40.138	25.713
Aydıncık Pond	P1	40.147	25.925
Şirinköy Pond	P2	40.114	25.772
Uğurlu Pond	P3	40.140	25.737

Figure 1. Topographic map of Gökçeada Island and selected locations

Health Risk Assessment

In the current research, population was divided into four age groups based on their physiological and behavioural differences as follow: infants (< 2 years old), children (2 – 6 years old), teenagers (6 – 16 years old) and adults (> 16 years old). The daily exposure to fluoride was calculated in these four different groups by using the equation 1. Hazard Quotient (HQ) describes the non-carcinogenic risk of fluoride and it was calculated by using the equation 2 (Yousefi et al., 2018). A lower value of HQ than 1 reflects a negligible risk of non-carcinogenic effects and a higher value of HQ than 1 reflects an important health risk and a significant risk of severe fluorosis may be present.

$$EDI = \frac{Cf \ x \ Cd}{Bw} \tag{1}$$

$$HQ = \frac{EDI}{RfD} \tag{2}$$

EDI: Estimation of fluoride consumption – daily (mg/kg/day)

C_f: Fluoride level in the investigated drinking water samples (mg/L)

C_d: Mean daily water intake (L/day) (the mean water consumption rates in infants, children, teenagers and adults were 0.08, 0.85, 2 and 2.5 L/day, respectively)

B_w: Body weight (kg) (body weights of investigated groups were considered 10, 15, 50 and 78 kg, respectively)

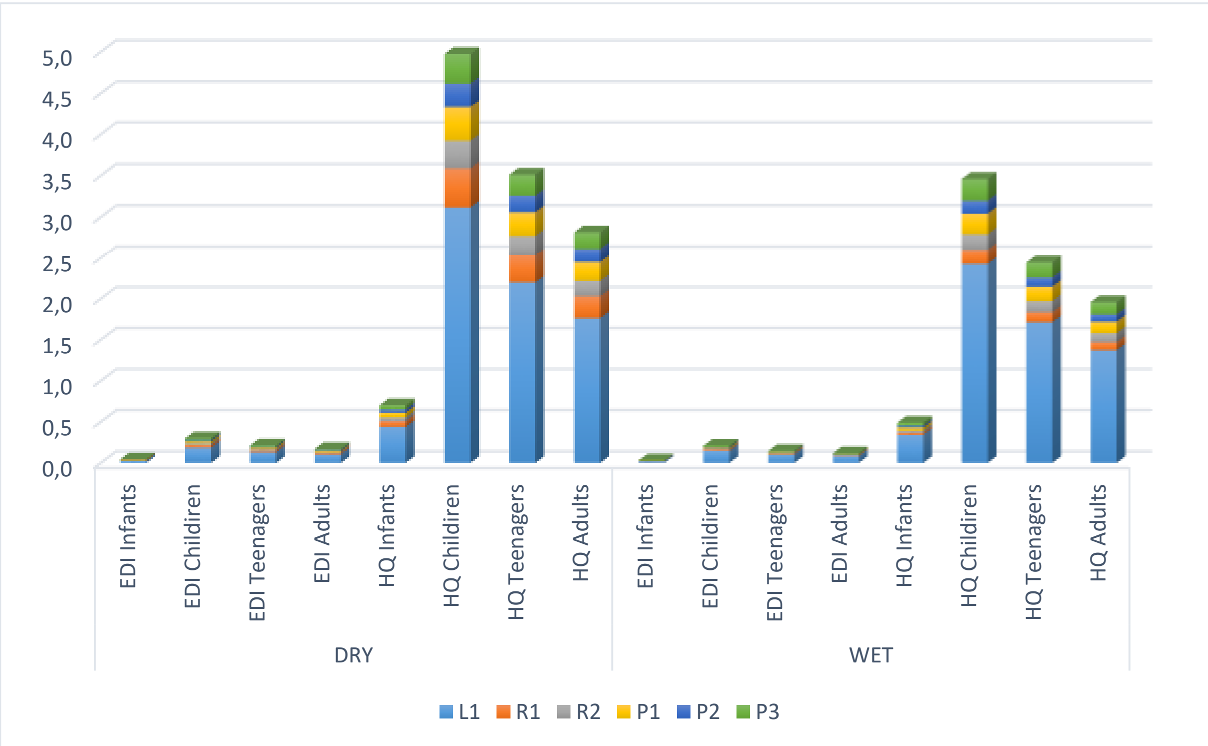
RfD: Reference dose (mg/kg/day) (it is 0.06 mg/kg/day for fluoride (EPA, 1992)).

RESULTS AND DISCUSSION

Spatial – temporal EDI – HQ values for the investigated different populations of consumers in water of natural and artificial lentic habitats of Gökçeada Island are given in Figure 2.

The average EDI values for fluoride in infants, children, teenagers and adults were recorded as 0.007, 0.050, 0.035 and 0.028 respectively in the dry season, while they were 0.005, 0.035, 0.024 and 0.020 respectively in the wet season. The average HQ values for fluoride in infants, children, teenagers and adults were recorded as 0.117, 0.829, 0.585 and 0.469 respectively, while they were 0.081, 0.577, 0.407 and 0.326 respectively in the wet season. The EDI and HQ risk rankings among the investigated different age groups were found as follows: children > teenagers > adults > infants in both dry and wet seasons in general.

In numbers of investigations on the assessment of health risks associated with fluoride in water conducted in many different countries confirmed that the children are at the chronic health risk due to the intake of fluoride in drinking water (Gao et al., 2013; Narsimha et al., 2018; Dehghani et al., 2019; Qasemi et al., 2020; Tokatlı et al., 2022; 2023). In the current research, as similar with the literature data, group of children were found as the riskiest age group for the health risk due to the intake of fluoride in water of natural and artificial lentic habitats of Gökçeada Island.



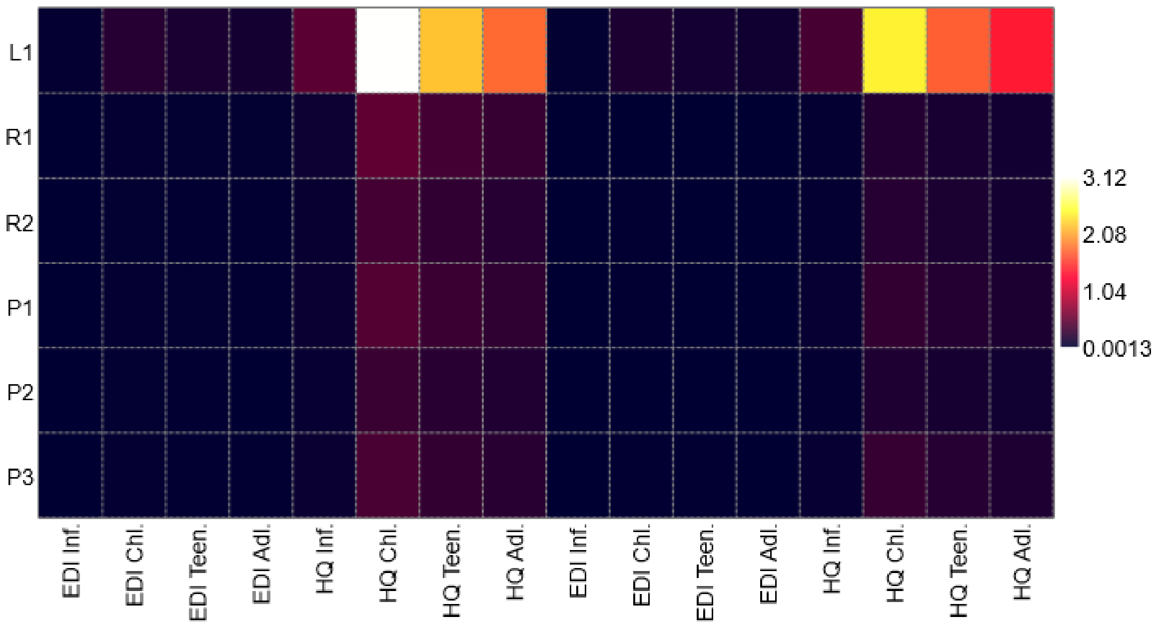


Figure 2. Spatio – temporal EDI – HQ values of different water consumers

CONCLUSIONS

In the current research, the non-carcinogenic health risks of fluoride via daily human intake in water of 6 lentic habitats located in Gökçeada Island of Türkiye were investigated. As a result of this study, the detected HQ values of all age groups were found as less than the critical limit of 1 and the risk rankings among the investigated different age groups were found as follows: children > teenagers > adults > infants in general. It was also determined that EDI and HQ values were significantly decreased in all the investigated natural and artificial lentic habitats during the rainy season.

ACKNOWLEDGEMENTS

This research was supported by Trakya University Scientific Research Projects (2022/168).

REFERENCES

Anonymous, 2022. 2022 Environmental status report of Çanakkale Province. Republic of Türkiye, Çanakkale Governorship, Provincial Directorate of Environment and Urban Management.

Dehghani, M. H., Zarei, A., Yousefi, M., Asghari, F. B., Haghighat, G. A., 2019. Fluoride Contamination in Groundwater Resources in the Southern Iran and Its Related Human Health Risks. Desalination Water Treatment, 153, 95–104.

Din, I. U., Wajid, A., Muhammad, S., Rehman, I., Tokatlı, C., 2024. Spatial Distribution and Potential Health Risk Assessment for Fluoride And nitrate Via Water Consumption in Pakistan. Journal Of Geochemical Exploration, 259: 107413.

EPA, 1992. Framework for Ecological Risk Assessment. Risk Assessment Forum. US Environmental Protection Agency, Washington, DC.

Gao, H. J., Jin, Y. Q., Wei, J. L., 2013. Health Risk Assessment of Fluoride in Drinking Water from Anhui Province in China. Environ. Monit. Assess., 185 (5), 3687–3695.

https://www.gokceada.bel.tr/

http://www.gokceada.gov.tr/

https://www.kulturportali.gov.tr/

Jannat, J. N., İslam, K. M. S., İslam, H. M. T., İslam, M. S., Khan, R., Bakar, S. M. A., Varol, M., Tokatlı, C., Chandra, P. S., İslam, A., İdris, A. M., Malafaia, G., Islam, A. R. T., 2022. Hydrochemical Assessment of Fluoride and Nitrate in Groundwater from East and West Coasts of Bangladesh and India. Journal of Cleaner Production, https://doi.org/10.1016/j.jclepro.2022.133675.

Narsimha, A., Rajitha, S., 2018. Spatial Distribution and Seasonal Variation in Fluoride Enrichment in Groundwater and Its Associated Human Health Risk Assessment in Telangana State, South India. Hum. Ecol. Risk Assess., 24 (8), 2119–2132.

Qasemi, M., Farhang, M., Morovati, M., Mahmoudi, M., Ebrahimi, S., Abedi, A., 2020. Investigation of Potential Human Health Risks from Fluoride and Nitrate Via Water Consumption in Sabzevar, Iran. Int. J. Environ. Anal. Chem., 1–12.

Tokatlı, C., Ustaoğlu, F., 2020. Health Risk Assessment of Toxicants in Meriç River Delta Wetland, Thrace Region, Turkey. Environmental Earth Science, 79: 426.

Tokatlı, C., Varol, M., 2021a. Variations, Health Risks, Pollution Status and Possible Sources of Dissolved Toxic Metal(loid)s in Stagnant Water Bodies Located in an Intensive Agricultural Region of Turkey. Environmental Research, 201: 111571.

Tokatlı, C., Varol, M., 2021b. Impact of the Covid-19 Lockdown Period on Surface Water Quality in the Meriç-Ergene River Basin, Northwest Turkey. Environmental Research, 197: 111051.

Tokatlı, C., İslam, A. R. T., Güner, Ş. O., Ustaoğlu, F., İslam, S., Dindar, M. B., 2022. A Pioneering Study on Health Risk Assessment of Fluoride in Drinking Water in Thrace Region of Northwest Türkiye. Groundwater for Sustainable Development, https://doi.org/10.1016/j.gsd.2022.100836.

Tokatlı, C., Onur, Ş. G., Dindar, M. B., Malafaia, G., Islam, A. R. T., Muhammad, S., 2023. Spatial-Temporal Variability and Probabilistic Health Risk Assessment of Fluoride from

Lentic Ecosystem, Türkiye. International Journal of Environmental Analytical Chemistry, https://doi.org/10.1080/03067319.2023.2198645.

Ustaoğlu, F., Aydın, H., 2020. Health Risk Assessment of Dissolved Heavy Metals in Surface Water in a Subtropical Rivers Basin System of Giresun (North-Eastern Turkey). Desalination and Water Treatment, 194. 222-234.

Varol, M., Tokatlı, C., 2022. Seasonal Variations of Toxic Metal(Loid)S in Groundwater Collected from an Intensive Agricultural Area in Northwestern Turkey and Associated Health Risk Assessment. Environmental Research, 204, Part A: 111922.

Yousefi, M., Ghoochani, M., Mahvi, A. H., 2018. Health Risk Assessment to Fluoride in Drinking Water of Rural Residents Living in the Poldasht City, Northwest of Iran, Ecotoxicol. Environ. Saf., 148: 426–430.

SPATIAL – TEMPORAL VARIATIONS OF FLUORIDE IN STAGNANT WATER BODIES OF GÖKÇEADA ISLAND THE WESTERNMOST PART OF TÜRKIYE: A GIS BASED ASSESSMENT

Mediha Büyükgöze Dindar[1], Meltem Tekbaş Atay[2], Şirin Güner Onur[3], Cem Tokatlı[4]*

[1]*Trakya University, Health Services Vocational School, Edirne / Türkiye*

[2]*Trakya University, Faculty of Dentistry, Edirne / Türkiye*

[3]*Altınbaş University, Faculty of Dentistry, İstanbul / Türkiye*

[4]*Trakya University, İpsala Vocational School, Edirne / Türkiye*

**Corresponding author: medihabuyukgoze@trakya.edu.tr*

ABSTRACT

Gökçeada, a district of Çanakkale, is the largest island in Türkiye. It is one of the rare islands in the world whose drinking water resources are sufficient to meet its own needs. There are 2 dam lakes, 3 artificial ponds and a lagoon on the island, which may use its fresh water resources for both drinking and irrigation. The aim of this research was (1) to determine the spatial – temporal variations of fluoride accumulations in water of stagnant water bodies located in the Gökçeada Island of Türkiye and (2) to evaluate the data in terms of dental health of the local people. 6 stagnant water bodies were selected on the island and water samples were collected during the dry – wet seasons of 2022 – 2023. The fluoride concentrations were measured by using spectrophotometric method. According to the results of this study, the fluoride accumulations were varied from 0.166 – 3.300 mg/L. It has been also noted that, the fluoride contents of water bodies decreases approximately 1.5 times during the rainy season, in general.

Keywords: Gökçeada Island, Stagnant Water Bodies, Fluoride Accumulations

INTRODUCTION

Fluoride is an essential element and it has a critical importance on the dental health. One of the main sources of fluoride intake is drinking water, but foods, drugs and industrial exposure are also among the entryways to the human body (Andezhath et al., 1993; Güner et al., 2016; Tokatlı and Güner, 2018; Onur et al., 2019; Onur and Tokatlı, 2020; Tokatlı et al., 2022; 2023).

Gökçeada, which has a very rugged landform due to its location on a volcanic ground, is one of the rare islands in the world whose drinking water resources are sufficient to meet its needs. When compared in terms of fresh water resources, it ranks first among the islands in the Aegean

Sea and fourth in the world. Gökçeada, which has 4 other ponds other than Zeytinli Dam Lake, may use its fresh water for both drinking and irrigation. Although Zeytinli Dam was built as a small dam, it provides most of the island's drinking water. There is a lagoon named as Salt Lake located in the northwest of the island. This lagoon was formed by the evaporation of the sea and condensation on land again and it is very important for natural life as it is located on the migration routes of migratory birds (Anonymous, 2022; http://www.gokceada.gov.tr/; https://www.gokceada.bel.tr/; https://www.kulturportali.gov.tr/).

The aim of this research was to determine the spatial – temporal variations of fluoride accumulations in water of stagnant water bodies located in the Gökçeada Island of Türkiye and to evaluate the data in terms of dental health of the local people.

MATERIALS AND METHODS

Water Collection

In this research, 6 stagnant water bodies (1 natural lake, 2 dam lakes and 3 artificial pons) located in the Gökçeada Island were selected. The coordinate information with the names of investigated freshwater ecosystems are given in Table 1. The topographic map of Gökçeada Island and selected locations are given in Figure 1. Water samples were collected in the summer season of 2022 (dry season) and the winter season of 2023 (wet season) with a telescopic water sampling device approximately 3 meters from the shore into the pre – cleaned polyethylene bottles.

Table 1. Coordinate information of selected stagnant water bodies

Name of Lentic	Station Code	GPS-North	GPS-East
Gökceada Lagoon	L1	40.133	25.956
Zeytinli Reservoir	R1	40.176	25.863
Uğurlu Reservoir	R2	40.138	25.713
Aydıncık Pond	P1	40.147	25.925
Şirinköy Pond	P2	40.114	25.772
Uğurlu Pond	P3	40.140	25.737

Figure 1. Topographic map of Gökçeada Island and selected locations

Fluoride Analysis

Fluoride levels of stagnant water bodies of Gökçeada Island were determined with spectrophotometric method by using a "Hach Lange DR 3900 Spectrophotometer" with a wavelength range of 320 – 1100 nm and "Hach Lange LCK 323 Cuvette Test" with a measure range of 0.1 – 2.5 mg/L (https://tr.hach.com/).

RESULTS AND DISCUSSION

Fluoride accumulations in water of stagnant water bodies of Gökçeada Island are given in Figure 2 as GIS based distribution maps and spatial – temporal variations of fluoride levels are given in Figure 3.

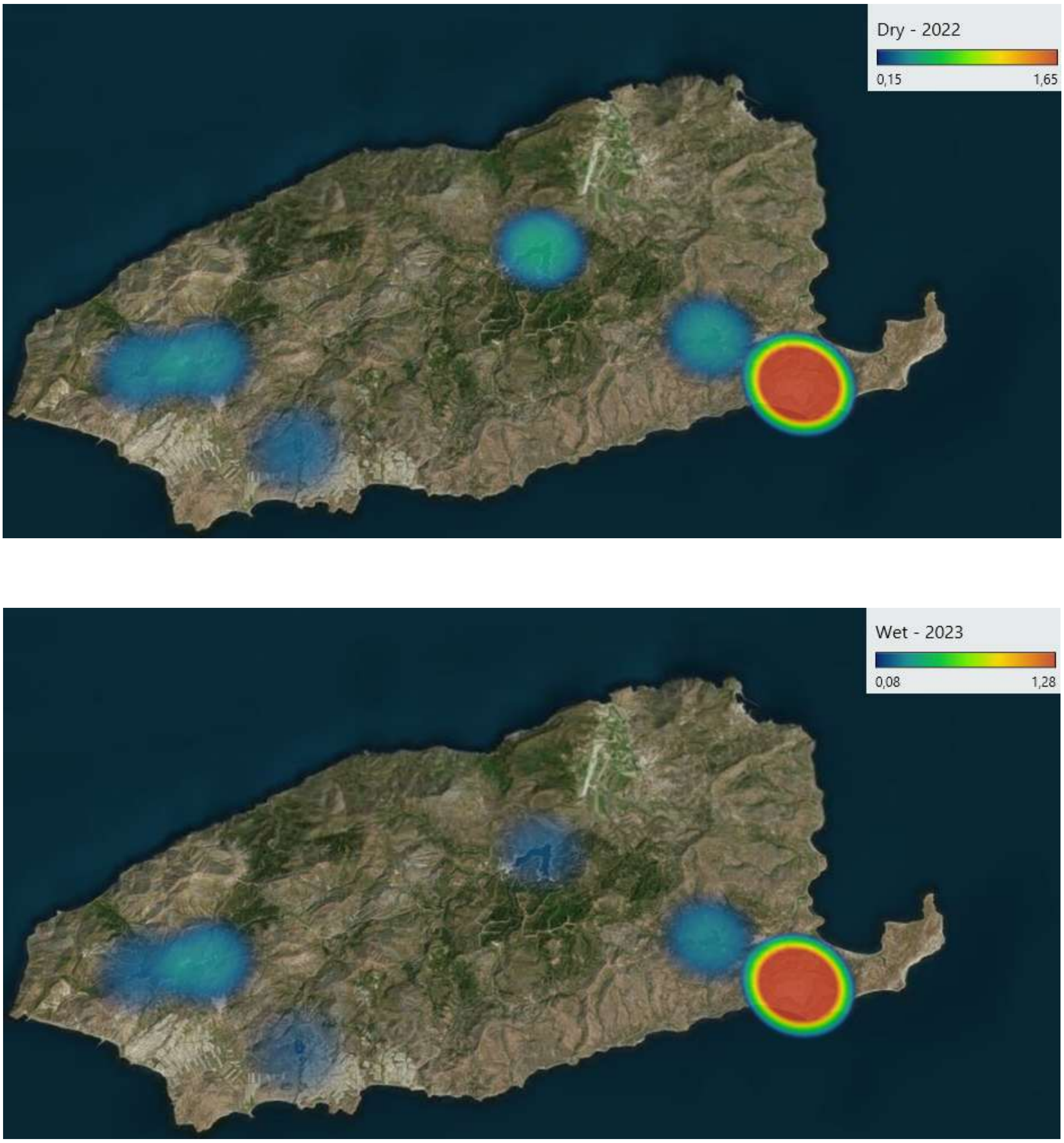


Figure 2. Fluoride levels of stagnant water bodies in dry (up) and wet (down) seasons (mg/L)

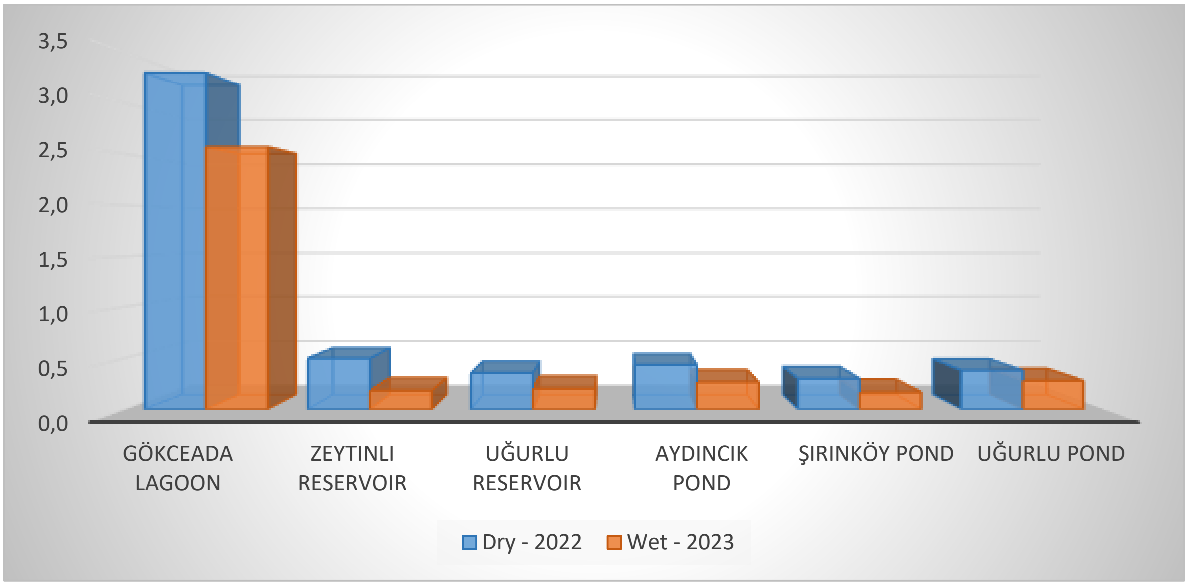


Figure 3. Spatial – temporal fluoride accumulations of stagnant water bodies (mg/L)

The fluoride accumulations in water of stagnant water bodies of Gökçeada Island varied from 0.300 mg/L (Şirinköy Pond) – 3.300 mg/L (Gökceada Lagoon) with a mean value of 0.878 mg/L in the dry season, while they were varied from from 0.166 mg/L (Şirinköy Pond) – 2.570 mg/L (Gökceada Lagoon) in the wet season with a mean value of 0.611 mg/L (Figure 3).

Although fluoride, which may easily get deposited in teeth, has a special importance in terms of especially dental health. It may have also quite adverse health effects on human depending on the content and consumption (Ayoob and Gupta, 2006; GómezHortigüela, 2013; Taghipour et al., 2016; Tokatlı et al., 2022; 2023). Drinking water is a direct source of fluoride exposure and urban water with optimal fluoride levels has preventive effect on body health and especially dental caries. However, exposure to fluoride more than optimal level may result in fluorosis that is an important dental health problem (Güner et al., 2016; Tokatlı and Güner, 2018; 2020; Jannat et al., 2022; Din et al., 2024). In the current research, the concentrations of fluoride in water of all the investigated stagnant water bodies located in the Gökçeada Island were ranged from 0.166 – 3.300 mg/L and fluoride levels of all the investigated 5 stagnant water bodies used as drinking water were within the acceptable limit of 1.5 mg/L for drinking (except Gökceada Lagoon – not used as drinking water) specified by TS266 (2005), EC (2007) and WHO (2011).

CONCLUSIONS

In the current research, spatial – temporal variations of fluoride accumulations of fluoride in water of 6 stagnant water bodies located in the Gökçeada Island of Türkiye were investigated. As a result of this study, the fluoride accumulations were varied as 0.300 – 3.300 mg/L in the dry season and 0.166 mg/L – 2.570 mg/L in the wet season. It was also determined that fluoride levels in waters were significantly decreased in all the investigated stagnant water bodies during the rainy season.

ACKNOWLEDGEMENTS

This research was supported by Trakya University Scientific Research Projects (2022/168).

REFERENCES

Andezhath, S. K., Kumar, A., Bhatnagar, M., Bahudur, R., 1993. Prevalence of Endemic Fluorosis with Gastro-Intestinal Manifestations in People Living in Some North-Indian Villages. Fluoride, 26 (2): 97-104.

Anonymous, 2022. 2022 Environmental status report of Çanakkale Province. Republic of Türkiye, Çanakkale Governorship, Provincial Directorate of Environment and Urban Management.

Ayoob, S., Gupta, A. K., 2006. Fluoride in Drinking Water: A Review on the Status and Stress Effects. Critical reviews in Environmental Science and Technology, 36. 433-487.

Din, I. U., Wajid, A., Muhammad, S., Rehman, I., Tokatlı, C., 2024. Spatial Distribution and Potential Health Risk Assessment for Fluoride And nitrate Via Water Consumption in Pakistan. Journal Of Geochemical Exploration, 259: 107413.

EC (European Communities), 2007. European Communities (drinking water) (no. 2). Regulatıons 2007. S.I. No. 278 of 2007.

GómezHortigüela, L., PérezPariente, J., García, R., Chebude, Y., Díaz, I., 2013. Natural zeolites from Ethiopia for elimination of fluoride from drinking water. Sep Purif Technol., 120:224–229.

Güner, Ş., Uyar-Bozkurt, S., Haznedaroğlu, E., Menteş, A., 2016. Dental Fluorosis and Catalase Immunoreactivity of the Brain Tissues in Rats Exposed to High Fluoride Pre- and Postnatally. Biol Trace Elem Res., 174 (1): 150-157.

https://www.gokceada.bel.tr/

http://www.gokceada.gov.tr/

https://tr.hach.com/

https://www.kulturportali.gov.tr/

Jannat, J. N., İslam, K. M. S., İslam, H. M. T., İslam, M. S., Khan, R., Bakar, S. M. A., Varol, M., Tokatlı, C., Chandra, P. S., İslam, A., İdris, A. M., Malafaia, G., Islam, A. R. T., 2022. Hydrochemical Assessment of Fluoride and Nitrate in Groundwater from East and West Coasts of Bangladesh and India. Journal of Cleaner Production, https://doi.org/10.1016/j.jclepro.2022.133675.

Onur, Ş. G., Sezgin, B. I., Tokatlı, C., Haznedaroğlu, E., Eda, A., İldeş, G. Ç., Kalaoğlu, E. E., Yazıcı, B., Menteş, A., 2019. Edirne'nin Içme Suyu Fluor Orani Farkli 3 Ilçesinde Dental Fluorozis Ve Diş Çürüğü Prevalansinin Değerlendirilmesi. Yeditepe Üniversitesi Diş Hekimliği Fakültesi Dergisi, 15 (2): 219-223.

Onur, Ş. G., Tokatlı, C., 2020. Comparison of Fluoride Contents in Terms of Teeth Health and Water Quality in Drinking Water at the Northern and Southern Regions of Meriç River Basin (Edirne/Turkey). International Journal of Agriculture. Environment and Food Sciences, 4 (2): 173-180.

Taghipour, N., Amini, H., Mosaferi, M., Yunesian, M., Pourakbar, M., Taghipour, H., 2016. National and Sub-National Drinking Water Fluoride Concentrations and Prevalence of Fluorosis and of Decayed, Missed and Filled Teeth in Iran from 1990 to 2015: A Systematic Review. Environmental Science and Pollution Research, 23 (6): 5077–5098.

Tokatlı, C., Güner, Ş., 2018. Fluorine Accumulations in Drinking Water of Havsa District (Edirne. Turkey) and Assessment of Water Quality in Terms of Teeth Health. Sigma Journal of Engineering and Natural Sciences, 36 (3): 887-894.

Tokatlı, C., Güner, Ş., 2020. Fluoride Levels in Drinking Water and Assessment of Water Quality in terms of Teeth Health in a Significant Watershed in Thrace Region. Acta Aquatica Turcica. 16 (2): 238-245.

Tokatlı, C., İslam, A. R. T., Güner, Ş. O., Ustaoğlu, F., İslam, S., Dindar, M. B., 2022. A Pioneering Study on Health Risk Assessment of Fluoride in Drinking Water in Thrace Region of Northwest Türkiye. Groundwater for Sustainable Development, https://doi.org/10.1016/j.gsd.2022.100836.

Tokatlı, C., Onur, Ş. G., Dindar, M. B., Malafaia, G., Islam, A. R. T., Muhammad, S., 2023. Spatial-Temporal Variability and Probabilistic Health Risk Assessment of Fluoride from Lentic Ecosystem, Türkiye. International Journal of Environmental Analytical Chemistry, https://doi.org/10.1080/03067319.2023.2198645.

TS 266, 2005. Sular-İnsani Tüketim Amaçlı Sular. Türk Standartları Enstitüsü. ICS 13.060.20.

WHO (World Health Organization), 2011. Guidelines for Drinking-water Quality. World Health Organization Library Cataloguing-in-Publication Data, NLM Classification: WA 675.

IMMUNE RESPONSE OF INSECT IN TERM OF *GALLERIA MELLONELLA*: AN ROBUST PHYSIOLOGICAL MODEL TO DEVELOP INSECTICIDAL DRUGS

Kübra Aslan[1,], Çağrı Alkan[2], Cihat Çelik[3], Ender Büyükgüzel[2], Kemal Büyükgüzel[1]*

[1] Zonguldak Bülent Ecevit University, Science Faculty, Dept of Biology, Zonguldak,, Türkiye
[2]Zonguldak Bülent Ecevit University Department of Molecular Biology and Genetics, Science Faculty, Zonguldak TR-67100, Türkiye
[3]Zonguldak Bülent Ecevit University, Çaycuma Food and Agriculture Vocational School, Department of Chemistry and Chemical Processing Technologies, Zonguldak,, Türkiye
Corresponding author email: kubraaslan7676@gmail.com

ABSTRACT

Immunity is crucial for protecting organisms from diseases and harmful substances. Vertebrates and insects have immune systems with different molecular mechanisms. Vertebrates, like humans, have innate and adaptive immunity. The innate response offers immediate, nonspecific defenses, including physical barriers like skin and mucous membranes, along with cellular and humoral responses involving various immune cells and soluble components. On the other hand, adaptive immunity entails highly specialized responses mediated by T and B cells that form memories against specific infections. However, insects, such as *Galleria mellonella* model rely heavily on their innate immune system for defense. This system, which includes physical barriers, cellular reactions, and humoral components, responds to invading pathogens in a quick and nonspecific manner. Cellular defenses in insects include nodulation, phagocytosis, encapsulation, the respiratory burst in which free radicals are generated, and humoral defense mechanisms such as prophenol oxidase, antimicrobial peptides, and coagulation. Furthermore, molecular pathways like Imd (Immune deficiency), mTOR, and JAK-STAT are critical in coordinating insect immune responses. These pathways control a variety of immunological processes, including pathogen identification, signal transduction, and immune effector synthesis. Understanding the complex molecular mechanisms of insect immunity is critical not only for understanding the underlying principles of host defense, but also for practical applications like pest control and disease management. Insights into insect immunity would led light to develop new drugs by crippling of insect immune system by immunosupressive innovative drugs such as eicosanoid (immune meditor molecules in insects) biosynthesis inhibitors for combating insect-borne diseases in human and agricultural pests while limiting environmental effects.

Keywords: Immune Response, Insects, *Galleria mellonella*, Immunosupressive Drugs

INTRODUCTION

The immune system in living organism’s functions as a mechanism of defence that is complex and essential for survival and adaptation (Parkin and Cohen, 2001). It includes both innate and adaptive components that develop over time to protect against infections and infections, parasitism, and harmful interactions (Danilova, 2006). Immunity also involves the identification and elimination of foreign genetic material through various mechanisms, including the restriction-modification and CRISPR-Cas systems (Zakrzewska and Burmistrz, 2023). Initiation of immune responses is facilitated by phagocytic macrophages that detect bacterial surfaces and secrete cytokines and chemokines to provoke inflammation and recruit additional immune cells (Arango and Descoteaux, 2014). The evolution of the immune system is closely linked to the broader evolutionary narrative of life on Earth, ranging from the origin of prokaryotic organisms to changes in multicellular size and the development of adaptive immune responses in vertebrates (Cooper and Alder, 2006). In summary, the immune system in living organisms is crucial for maintaining the integrity and homeostasis of organisms, emphasising the importance of these complex evolutionary processes.

Innate Immunity in Vertebrates

The innate immune system in vertebrates is the primary line of defence against pathogens. This immune system functions as a physical barrier as well as cellular and humoral responses (Kasamatsu, 2013). Since physical barriers such as skin and mucosa are the first points of contact with danger, epithelial cells that prevent infection here are extremely important. Mucus formed by epithelial cells secretes substances such as perspiration and saliva, creating a potential defence mechanism against pathogens (Gallo and Hooper, 2012). It acts as natural killer cells and these tissues have added an important feature to the innate immune system by exerting microcidal effects against viruses and gaining memory cell properties with interleukins 12 and 18 (Kyndra, 2022). The cellular response needs to produce interferons and pro-inflammatory cytokines to fight viral infections and interferon lambda is crucial in antiviral immune responses (Liu et al., 2024). To understand these immune mechanisms, it is crucial to study the innate immune system of vertebrates in the evolutionary process (Kasamatsu, 2013).

Insects have strong immune systems that include physical and more different immune responses against pathogens (Zhou et al., 2024). Since insects do not have an innate immune system like vertebrates, they show different immune responses to survive against pathogens (Hoffmann and Hetru, 1992). In the insect immune system, humoral immunity has a very important influence on the metabolic adaptation and behaviour of insects (Nunes et al., 2021). In insect immunity, haemocytes, phagocytosis, nodulation and encapsulation processes play an important role in cellular and humoral responses (Marmaras and Lampropoulou, 2009). These responses are interconnected in insect immunity. While haemoxides affect humoral factors, they form a strong defence system against pathogens. When insect immunity is viewed from a broad perspective, the immune system of insects is sensitively tuned for survival and adaptation to various environmental conditions.

Adaptive Immunity in Vertebrates

Adaptive immunity in vertebrates is mainly determined by the primary functions of T and B cells in the formation of immunological memory. T and B lymphocytes undergo extensive genetic regulation to produce a variety of antigen-specific receptors that enable recognition of many pathogens. Following initial exposure to an antigen, these cells undergo proliferation as well as differentiation, leading to the formation of long-term memory lymphocytes that can respond quickly and effectively upon subsequent exposure to the same pathogen (Yang et al., 2021). The development of memory T cells is modulated by many different factors, including the balance between memory formation and immunoregulation, which can influence the outcome of infectious diseases, cancer and autoimmune diseases (Kurtz and Franz, 2003). Similarly, the process of memory formation, particularly in B cells, is complex and requires strategic choices regarding the specificity and affinity of immune receptors, which are vital for maintaining effective long-term immunity (Netea et al., 2019). It is also effective in generating immediate responses at barrier sites to enable tissue-resident B cells to provide defence (Cancro and Tomayko, 2021). In short, the memory-forming ability of the adaptive immune system is vital for the persistent survival in vertebrates against a variety of pathogens.

Comparison Between Vertebrate and Insect Immune Systems

The immune systems of vertebrates and insects have both similarities and differences. In Vertebrates, lymphocytes and immunoglobulins are highly functional, indicating the presence of both innate and adaptive immunity (Melillo et al., 2018). In contrast, insects rely solely on innate immunity to combat pathogens, using cellular and humoral mechanisms such as phagocytosis, secretion of antimicrobial peptides and activation of signalling pathways, including Toll and similar pathways (Simona and Jean-Luc, 2016). Whereas vertebrates with adaptive immunity function as lymphocytes equipped with specific antigen receptors, invertebrates, including insects and jawless vertebrates, have shown immune responses similar to adaptive immunity, thus challenging the traditional categorisation of immune systems (Shaochun et al., 2014). Moreover, while arthropods exhibit a wide variety of immune components with some elements resembling those found in vertebrates, such as Toll-like receptors, they also exhibit distinctive features such as significant gene duplication and variability in immune receptors (William et al., 2014). These observations underline that the immune systems of various animal taxons are more complex and have evolved in different directions in the evolutionary process and that there are diverse immune systems.

Insect Immune System

Insects show an immune system that encompasses both physical and humoral defence mechanisms. The first line of defence against pathogens is formed by physical barriers, including the trophic matrix and chitinous exoskeleton (Ulrich et al., 2022). Cellular defence mechanisms such as nodulation, phagocytosis, encapsulation and respiratory blast play a crucial role in the identification and inactivation of invading microorganisms (Hoffmann, 2003). The humoral immune response is primarily driven by the antimicrobial peptides (AMPs) and the prophenol oxidase pathway, which work to eliminate pathogens and facilitate blood clotting (Li et al., 2024; Ulrich et al., 2022). Recent research has underlined the complex nature of these immune responses, showing that the environmental factors can greatly influence their efficiency (Moure et al., 2022). Moreover, the interplay between various immune pathways, including the production of reactive oxygen species and associated signalling pathways, underlines the adaptive properties of insect immunity (Hoffmann, 2003; Ulrich et al., 2022) This overarching strategy enables insects to effectively counter a variety of pathogens, underlining their evolutionary success in immune defence.

Immune Response of Insect in Term of *Galleria mellonella*

G. mellonella displays a diverse immune system response. In recent years, there has been a large number of studies focusing on the immune mechanisms of this model organism. Larvae infection with *Pseudomonas entomophila* activates immune-related genes and defence strategies in a dose-dependent manner, leading to antimicrobial activity in the haemolymph (Jakub et al., 2023). While ink injections promote hemocyte mobilisation, lemongrass essential oil and citral have been found to inhibit this process (Kyndra et al., 2022). The melanisation process plays an important role in the immune response, as melanin encapsulation shows antifungal properties against Cryptococcus neoformans and other fungal pathogens (Trevijano and Zaragoza, 2018). *G. mellonella* is a very valuable model to research host-pathogen interactions and to evaluate therapies such as antimicrobial photodynamic therapy that are effective against various pathogens (Chastain et al., 2022). These observations emphasise the importance of *G. mellonella* in the study of immune responses and its potential for various biological applications.

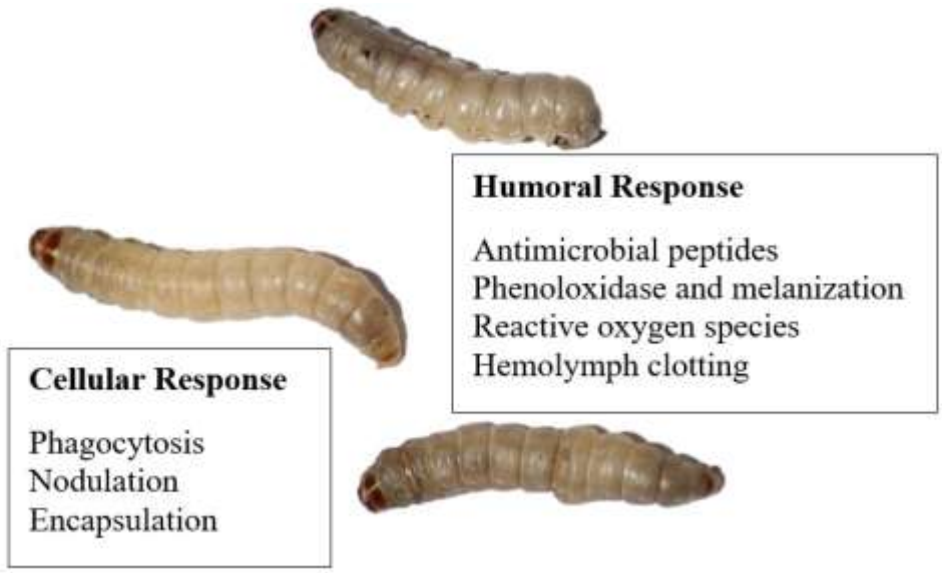


Figure 1. *G. mellonella* immune response (The photograph of the *Galleria mellonella* 7th stage larva was taken by our team).

Pathways in Insect Immunity

Insect immunity is regulated by several critical molecular pathways, in particular the Imd, mTOR and JAK-STAT pathways. The Imd pathway is crucial for generating immune responses against Gram-negative bacteria, as it triggers the synthesis of antimicrobial peptides (AMPs) through specific receptors that detect pathogen-associated molecular patterns (Lovett and Leger 2017; Ulrich et al., 2022). The evolutionarily conserved JAK-STAT pathway is important in modulating immune responses against various pathogens, including viruses and fungi, by mediating cytokine signalling and enhancing immune cell differentiation (Xiang-Yi et al., 2023; Krishnakumar and Kannan, 2020). Furthermore, the mTOR pathway is involved in cellular growth and metabolism and influences immune responses by integrating signals from both nutrients and pathogens (Kurtz and Franz, 2003). Collectively, these pathways contribute to a strong immune response; however, pathogens have evolved strategies to circumvent these defences, which has led to ongoing research into the intricacies of insect immunity and the influence of non-coding RNAs on these pathways ((Kurtz and Franz, 2003; Ulrich et al., 2022). However, gaining insight into these interactions is vital for formulating new approaches to enhance insect resistance to pathogens.

Applications of Insect Immunity Research

Research in the field of insect immunology has significant potential for the development of pest control methods, management of agricultural diseases and the creation of immunosuppressive pharmaceuticals. A comprehensive understanding of the mechanisms underlying insect innate immunity, including the functions of antimicrobial peptides (AMPs) and immune signalling pathways, could lead to the development of novel pest management strategies. For example, the distinctive immune responses observed in aphids offer potential targets for environmentally friendly pest control by exploiting their natural defences against various pathogens and parasites (Yang et al., 2021) Furthermore, the identification of AMPs as natural antimicrobial agents provides valuable opportunities to support food safety and improve agricultural productivity (Moure et al., 2022). In addition, investigating the immunomodulatory roles of non-coding RNAs could pave the way for the creation of immunosuppressive drugs that could have applications in both agricultural and medical fields (Lovett and Leger, 2017). In summary, integrating insect immunity research into practical applications offers promising prospects for sustainable agricultural practices and improved health outcomes (Moure et al., 2022; Lovett and Leger, 2017).

CONCLUSIONS

Understanding the immune mechanisms of vertebrates and insects reveals the diversity and specialisation of their defence strategies. Vertebrates, like humans, have both innate and adaptive immune systems, allowing for immediate, non-specific responses mediated by T and B cells and highly specific, long-lasting immunity. In contrast, insects such as *G. mellonella* rely primarily on an evolved innate immune system. This system includes physical barriers, cellular defences such as phagocytosis and encapsulation, and humoral responses such as antimicrobial peptides and the prophenol oxidase pathway. Key molecular pathways such as Imd, mTOR and JAK-STAT play important roles in regulating insect immune responses, including pathogen recognition, signal transduction and production of immune effectors. By delving deeper into the complexities of insect immunity, we are not only improving our understanding of the fundamentals of host defence, but also paving the way for innovative applications in pest control and disease management. Targeting the insect immune system with immunosuppressants such as eicosanoid biosynthesis inhibitors offers a promising approach to combat insect-borne diseases and agricultural pests. This strategy has the potential to minimise environmental impacts while effectively reducing the prevalence of these challenges. Therefore, continued research on insect immunity is essential to develop new and sustainable solutions to global agricultural and health challenges.

REFERENCES

Arango Duque, G., and Descoteaux, A. 2014. Macrophage cytokines: involvement in immunity and infectious diseases. Frontiers in immunology, 5, 491. https://doi.org/10.3389/fimmu.2014.00491

Cancro, M. P., and Tomayko, M. M. 2021. Memory B cells and plasma cells: The differentiative continuum of humoral immunity. Immunological reviews, 303(1), 72–82. https://doi.org/10.1111/imr.13016

Chastain, K., Peterson, W., Haszcz, K., Fenske, M., Rice, J., and Pszczolkowski, M. A. 2022. Innate Immune Response of *Galleria mellonella* (Lepidoptera: Pyralidae) Larvae to Lemongrass Essential Oil and Citral. Journal of Entomological Science, 57(4), 573-586.

Cooper, M. D., and Alder, M. N. 2006. The evolution of adaptive immune systems. Cell, 124(4), 815–822. https://doi.org/10.1016/j.cell.2006.02.001

Danilova, N. 2006. The evolution of immune mechanisms. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution, 306(6), 496-520.

Gallo, R. L., and Hooper, L. V. 2012. Epithelial antimicrobial defence of the skin and intestine. Nature reviews. Immunology, 12(7), 503–516. https://doi.org/10.1038/nri3228

Hoffmann, J. A., and Hetru, C. 1992. Insect defensins: inducible antibacterial peptides. *Immunology today*, *13*(10), 411–415. https://doi.org/10.1016/0167-5699(92)90092-L

Jakub, Kordaczuk., Michał, Sułek., Paweł, Mak., Monika, Hułas-Stasiak., Iwona, Wojda. 2023. Defence response of *Galleria mellonella* larvae to oral and intrahemocelic infection with Pseudomonasentomophila. Developmental and Comparative Immunology, https://doi.org/10.1016/j.dci.2023.104749

Kasamatsu J. 2013. Evolution of innate and adaptive immune systems in jawless vertebrates. Microbiology and immunology, 57(1), 1–12. https://doi.org/10.1111/j.1348-0421.2012.00500.x

Krishnakumar, V., Kannan, M. 2020. Microbial Pathogenesis: Virus Pathogen–Host Interactions. In: Siddhardha, B., Dyavaiah, M., Syed, A. (eds) Model Organisms for Microbial Pathogenesis, Biofilm Formation and Antimicrobial Drug Discovery. Springer, Singapore. https://doi.org/10.1007/978-981-15-1695-5_2

Kurtz, J., and Franz, K. 2003. Evidence for memory in invertebrate immunity. Nature, 425(6953), 37–38. doi:10.1038/425037a

Kyndra, Chastain., Westley, Peterson., Katherine, Haszcz., Michaela, Fenske., Jennifer, Rice., Maciej, A., Pszczolkowski. 2022. Innate Immune Response of *Galleria mellonella* (Lepidoptera: Pyralidae) Larvae to Lemongrass Essential Oil and Citral. Journal of Entomological Science, https://doi.org/10.18474/JES22-08

Liu, Y. G., Jin, S. W., Zhang, S. S., Xia, T. J., Liao, Y. H., Pan, R. L., Yan, M. Z., and Chang, Q. 2024. Interferon lambda in respiratory viral infection: immunomodulatory functions and antiviral effects in epithelium. Frontiers in immunology, 15, 1338096. https://doi.org/10.3389/fimmu.2024.1338096

Li-Zhen, Zhou., Guanliang, Meng., Ling, Zhu., Li, Ma., Kangkang, Chen. 2024. Insect Antimicrobial Peptides as Guardians of Immunity and Beyond: A Review. International Journal of Molecular Sciences, doi: 10.3390/ijms25073835

Lovett, B., and Leger, R. J. S. 2017. The insect pathogens. The Fungal Kingdom, 923-943. Hoffmann J. A. (2003). The immune response of Drosophila. Nature, 426(6962), 33–38. https://doi.org/10.1038/nature02021

Marmaras, V. J., and Lampropoulou, M. 2009. Regulators and signalling in insect haemocyte immunity. Cellular signalling, 21(2), 186–195. https://doi.org/10.1016/j.cellsig.2008.08.014

Melillo, D., Marino, R., Italiani, P., and Boraschi, D. 2018. Innate Immune Memory in Invertebrate Metazoans: A Critical Appraisal. Frontiers in immunology, 9, 1915. https://doi.org/10.3389/fimmu.2018.01915

Moure, U. A. E., Tan, T., Sha, L., Lu, X., Shao, Z., Yang, G., ... and Cui, H. 2022. Advances in the immune regulatory role of non-coding RNAs (miRNAs and lncRNAs) in insect-pathogen interactions. Frontiers in Immunology, 13, 856457.

Netea, M. G., Schlitzer, A., Placek, K., Joosten, L. A. B., and Schultze, J. L. 2019. Innate and Adaptive Immune Memory: an Evolutionary Continuum in the Host's Response to Pathogens. Cell host and microbe, 25(1), 13–26. https://doi.org/10.1016/j.chom.2018.12.006

Nunes, C., Sucena, É., and Koyama, T. 2021. Endocrine regulation of immunity in insects. The FEBS journal, 288(13), 3928–3947. https://doi.org/10.1111/febs.15581

Parkin, J., and Cohen, B. 2001. An overview of the immune system. *The Lancet*, *357*(9270), 1777-1789.

Shaochun, Yuan., Xin, Tao., Shengfeng, Huang., Shangwu, Chen., Anlong, Xu. 2014. Comparative Immune Systems in Animals. Annual Review of Animal Biosciences, doi: 10.1146/ANNUREV-ANIMAL-031412-103634

Simona, Paro., Jean-Luc, Imler. 2016. Immunity in Insects. doi: 10.1016/B978-0-12-374279-7.12006-5

Trevijano-Contador, N., and Zaragoza, O. 2018. Immune response of *Galleria mellonella* against human fungal pathogens. *Journal of Fungi*, *5*(1), 3.

Ulrich, Aymard, Ekomi, Moure., Ting, Xu, Tan., Lina, Sha., Xiaoqin, Lu., Zhiyong, Shao., Guang, Yang., Yi, Wang., Hongjuan, Cui. 2022. Advances in the Immune Regulatory Role of Non-Coding RNAs (miRNAs and lncRNAs) in Insect-Pathogen Interactions. Frontiers in Immunology, doi: 10.3389/fimmu.2022.856457

William, J., Palmer., Francis, M., Jiggins. 2014. Comparative genomics reveals the origins and diversity of arthropod immune systems. bioRxiv, doi: 10.1101/010942

Xiang-Yi, Wei., Dong-Chun, Hu., Zu-Peng, Gao., Cong-Jing, Feng. 2023. JAK/STAT signaling pathway and its regulation on insect immunity. doi: 10.16288/j.yczz.22-402

Yang, L., Qiu, L. M., Fang, Q., Stanley, D. W., and Ye, G. Y. 2021. Cellular and humoral immune interactions between Drosophila and its parasitoids. Insect Science, 28(5), 1208-1227.

Zakrzewska, M., and Burmistrz, M. 2023. Mechanisms regulating the CRISPR-Cas systems. Frontiers in microbiology, 14, 1060337. https://doi.org/10.3389/fmicb.2023.1060337

Zhou, L., Meng, G., Zhu, L., Ma, L., and Chen, K. 2024. Insect Antimicrobial Peptides as Guardians of Immunity and Beyond: A Review. International journal of molecular sciences, 25(7), 3835. https://doi.org/10.3390/ijms25073835

ROLES OF EICOSANOIDS IN INSECT IMMUNE RESPONSE: CHALLENGING MOLECULES IN INSECTS

Çağrı ALKAN[1,], Cihat ÇELİK[2], Kübra ASLAN[3], Ender BÜYÜKGÜZEL[1], Kemal BÜYÜKGÜZEL[3]*

[1]Zonguldak Bülent Ecevit University Department of Molecular Biology and Genetics, Science Faculty, Zonguldak TR-67100, Türkiye
[2]Zonguldak Bülent Ecevit University, Çaycuma Food and Agriculture Vocational School, Department of Chemistry and Chemical Processing Technologies, Zonguldak TR-67900, Türkiye
[3]Zonguldak Bülent Ecevit University Department of Molecular Biology and Genetics, Science Faculty, Zonguldak TR-67100, Türkiye
Corresponding author e-mail: cagrialkan40@gmail.com

ABSTRACT

Eicosanoids are a large class of compounds known as intercellular signaling molecules derived from fatty acids with 20 carbon atoms. Eicosanoids were discovered in vertebrates as physiolohical mediators in the 1960s, and were shown to be present in insects in the 1980s. In both vertebrates and invertebrates, eicosanoids play important roles in the immune system as well as normal physiological roles such as digestion, reproduction and nervous system functions and also were secreted in pathophysiological process. Insects, unlike vertebrates, have an innate immune system and are not capable of developing adaptive immunity. The innate immune system of insects provides defense by forming physical barriers (integument, peritrophicmembrane), cellular and humoral immune responses and reactive free radicals (ROS and nitrous oxide radicals). Eicosanoids play a critical role in regulating and activating these immune responses via certain signal pathways. For example, prostaglandins and thromboxanes promote phagocytosis and respiratory burst against pathogens, while leukotrins and lipokines trigger inflammation and migration of immune cells. In conclusion, eicosanoids play a crucial role in the functioning of the insect immune system and are critical in defense against pathogens. Further research on the insect physiology and pathology of these molecules may offer new opportunities for insecticide development and pest control strategies. We foster to develop new drugs in term of agricultural insecticidal apporoaches by inhibiting eicosanoid biosynthesis in different tissue of insects by eicosanoid biosynthesis inhibitors (EBIs) such as Cyclooxygenases enzyme systems (COX-1 and 2), Lipoxygenases (LOXs), Epoxyeicosatrienoic acids (EETs) pathways in our laboratory.

Key Words: Eicosanoids, Insects, Immune Response, Eicosanoid Biosynthesis Inhibitors,

INTRODUCTION

One of the systems essential for the survival of organisms is the immune system, a defense system that protects against pathogens. This complex system has evolved over thousands of years to counter threats ranging from bacteria and viruses to fungi and parasites. Immune responses can be categorized into two major groups: innate or adaptive. Generally, organisms use mainly the rapid and non-specific mechanisms of the innate immune system to provide initial protection (Tunaz, 2004).

Insect Immunity

Insects have a highly effective innate immune system, although they lack the antibody-mediated adaptive immunity of vertebrates. This system, which has evolved over millions of years of evolution, is divided into two main branches: humoral and cellular immunity. Humoral immunity includes the production and secretion of antimicrobial peptides (AMPs) into the hemolymph (circulating fluid). These peptides, such as cecropins, atakins and defensins, show broad activity against bacteria, fungi and even viruses (Gillespie et al., 1997). Cellular immunity reacts by direct interaction between circulating hemocytes and pathogens (Strand and Pech, 1995). The main cellular responses include phagocytosis, nodulation, encapsulation and melanization (Strand and Pech, 1995).

Phagocytosis, the import and digestion of small-sized invaders into cells, is a fundamental defense mechanism that has been conserved throughout evolution (Stuart and Ezekowitz, 2005). Insect hemocytes, acting like mammalian macrophages, enclose pathogens in specialized compartments called phagosomes and enzymatically degrade them (Pearson et al., 2003). A more complex cellular process, nodulation, occurs when the numbers of invaders become too large for hemocytes to handle as a single cell (Satyavathi et al., 2014). In this process, hemocytes aggregate around invaders to form nodules that melanize and are isolated from the rest of the organism. Encapsulation similarly targets larger invaders, such as parasitoid eggs that are too large to be phagocytosed (Carton et al., 2002). Hemocytes form multilayered capsules around invaders, effectively isolating them from the organism. Melanization, a crucial process in both nodulation and encapsulation, involves the phenoloxidase (PO) enzyme system (Cerenius et al., 2008). This enzyme catalyzes the production of melanin, a toxic pigment that both physically encapsulates invaders and produces cytotoxic and cytotoxic products that contribute to their elimination (Nappi and Christensen, 2005). The insect immune system, including these mechanisms, is a very effective defense system against pathogens.

Insect And Mammalian Immunity

Although the innate immunity of insects and mammals has some differences, there are striking similarities, particularly in the components of humoral immunity (Sheehan et al., 2018). Both systems depend on the recognition of pathogen molecules by specialized receptors (Akira et al., 2006). Activation of these receptors triggers signaling cascades leading to the production of effector molecules, including AMPs, in both systems (Imler and Zheng 2004). For example, the Toll pathway in insects and the Toll-like receptor (TLR) pathway in mammals activate the transcription factor NF-κB, which plays an important role in regulating the expression of immune genes (Silverman and Maniatis 2001). This homology links to the IMD pathway in insects and the TNF-α pathway in mammals, both of which also activate NF-κB (Kleino and Silverman 2014).

Despite these similarities, insect and mammalian immunity also shows striking differences. Most obviously, insects do not have antibody-mediated adaptive immunity. While vertebrates have B and T lymphocytes that can establish highly specific and long- term immune responses, insects have defenses based only on innate immunity (Flajnik and Kasahara 2010). This lack of adaptive immunity is compensated for by the extraordinary diversity of insect AMPs and the production of thousands of unique receptors by alternative spilicing, such as DSCAM, thereby broadening the spectrum of pathogens that can be recognized (Schmucker et al., 2000). Furthermore, while mammalian melanization is primarily involved in skin pigmentation and UV protection, insect melanization plays a crucial role in innate immunity as described above (Brenner and Hearing, 2008, Nappi and Christensen, 2005). Finally, insect AMPs are primarily produced in adipose tissue, whereas mammalian AMPs are usually produced locally by cells at the site of infection (Bozzaet al., 2011, Zhang et al., 2021).

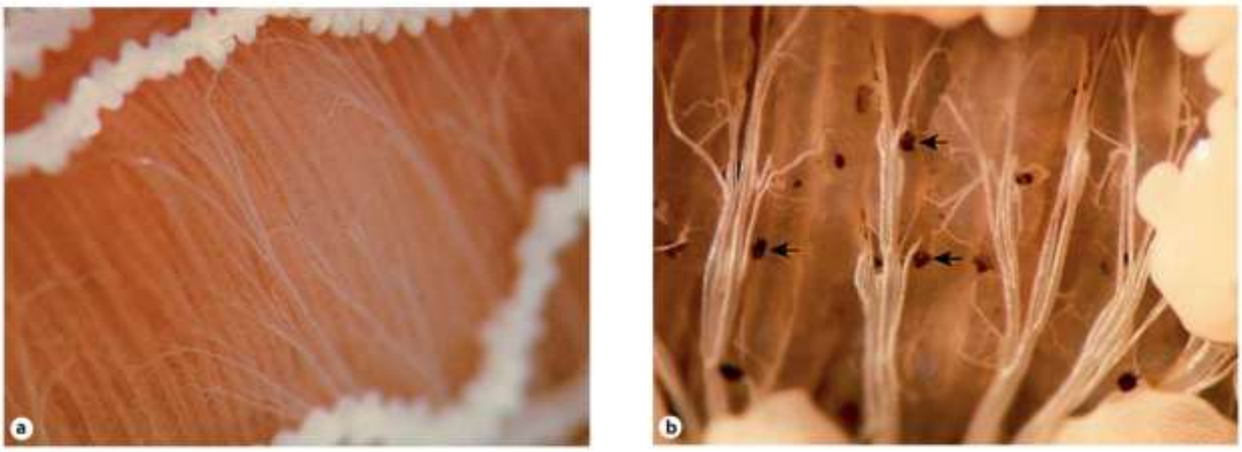


Figure 1. Nodulation reactions to bacterial infection in fifth instar tobacco hornworms, *Manduca sexta*. a) An immunologically naïve horn-worm. b) A hornworm following challenge by injecting the bacterium, *Serratia marcescens*, into the hemocoel. (Stanley et al., 2009).

HEMOLYMPH AND HEMOCYTES

Hemolymph is the circulating fluid that circulates in hemocele. It is in direct contact with the tissues. It is composed of hemocytes and plasma. The main functions of hemolymph are transport, storage and immunity. (Chapman 1998). The functions and structures of some hemocytes are listed below.

a. The prohemocyte: a stem cell. Other hemocytes are derived from prohemocytes. They are small cells with a small volume of cytoplasm. In many insect species, prohemocytes are located in the hematopoietic organs. A lower percentage are located in circulating hemolymph.

b. The granulocyte: contains a large number of granules. They are phagocytically active. After being activated by incoming impulses, granulocytes release granule contents into the surrounding environment. Garnulocytes are the most common type of hemolymph detected in Lepidopterans.
c. The plasmatocyte: are cells characterized by a high volume of cytoplasm that adheres strongly to surfaces. Plasmatocytes have been detected in lepidopterans. However, in some insect groups, including the genus Anopheles and Aedes, they have not been detected significantly.
d. The oenocytoid: it is a hemocyte that displays phenoloxidase activity. However, it does not show adhesion properties.

e. The spherule: cells with large spherules in the cytoplasm. They are responsible for the synthesis of cuticle components. (Chapman 1998).

Table 1. Similarities between insect hemocytes and human neutrophils (Browne et al., 2013).

	Hemocytes	Neutrophils
Phagocytosis	Lectin-mediated	Lectin-mediated
ROS	$O_2^{\cdot-}$, H_2O_2, $NO^{\cdot}$	$O_2^{\cdot-}$, H_2O_2, $NO^{\cdot}$
Degranulation	Yes	Yes
AMPs	Peroxynectin, transferrin, lysozyme, defensin	MPO, transferrin, lysozyme, defensin
Receptors	TLRs, B-1,3-glucan, IL-IR	TLRs, B-1,3-glucan, IL-IR
Transcription factors	NFκB, IκB	NFκB, IκB
Cascades	IMD, JNK, JAK-STAT	IMD, JNK, JAK-STAT
Kinases	p38 MAPK, ERK, PKC, PKA	p38 MAPK, ERK, PKC, PKA
Neutrophil extracellular nets (NET)	NET-like structures present	NETs present

EICOSANOIDS

Eicosanoids are molecules formed by enzymatic oxygenation of arachidonic acid and other 20-carbon polyunsaturated fatty acids. Eicosanoids are involved in many areas in the cell where they are synthesized. The main eicosanoids are leukotrienes, thromboxanes, prostacyclin, prostaglandins, lipoxins and hepoxylins (Stanley et al., 2009, Tunaz et al., 2018).

It has been reported that eicosanoids are involved in the formation of the immune response against bacteria in insects (Miller et al., 2005). Many studies with eicosanoid inhibitors have reported that eicosanoids are involved in insect immunity against infections (Stanley and Miller, 2006; Satyavathi et al., 2014, Tunaz et al., 2018).

Synthesis of Eicosanoids

Eicosanoid synthesis starts with the hydrolysis of arachidonic acid from the plasma membrane by Phospholipase A2. Hydrolyzed arachidonic acid is catalyzed to eicosanoid by 3 potential pathways. The first of these pathways is the cyclooxygenase pathway, which results in the formation of prostaglandins. Examples of prostaglandins are thromboxanes and prostacyclins. Another pathway is the lipooxygenase pathway. At the end of this pathway, a large group of eicosanoids are synthesized. This group includes leukotrienes, lipoxins, hepoxylins, peroxy-fatty acids and hydroxy-fatty acids. The last pathway is the cytochrome p450-epoxygenase pathway. As a result of this pathway, epoxyeicostrienoic acids are formed (Stanley et al., 2009).

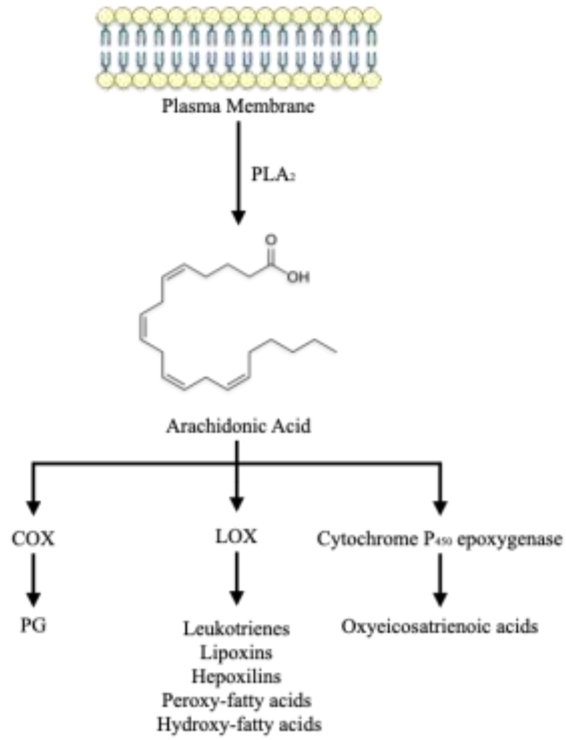


Figure 2. Eicosanoids synthesis. PLA_2: Phospholipase A_2, COX: cyclooxygenase, LOX: lipoxygenase, PG: prostaglandin.

CONCLUSIONS

Eicosanoids are molecules that enzymatically synthesized from polyunsaturated fatty acids by oxygenation reactions. They plays critical roles in several processes in insect immunity. These processes include migration of hemocytes, phagocytosis, synthesis of antimicrobial peptides. Studies have shown that these processes are affected when COX inhibitors, one of the enzymes responsible for eicosanoid synthesis, are used (Stanley-

Samuelson et al., 1991). Similarly, Shrestha and Kim in 2008 showed that prophenoloxidase activity is controlled by eicosanoids. In another study with eicosanoid synthesis inhibitors, it was observed that hemocyte migration was affected in a dose-dependent manner (Merchant et al., 2008). In addition, it has been shown the role of eicosanoids in the immune response to viral infections in *G. mellonella* (Büyükgüzel et al., 2007). Eicosanoids are also associated with the IDM and Toll pathways. Both pathways lead to the biosynthesis of eicosanoids by activating PLA2. In this case, eicosanoids have been shown to play an active role in cellular and humoral immunity (Satanley and Kim 2019).

REFERENCES

Akira, S. 2006. TLR signaling. *From innate immunity to immunological memory*, 1-16.

Bozza, P. T., Bakker-Abreu, I., Navarro-Xavier, R. A., Bandeira-Melo, C. 2011. Lipid body function in eicosanoid synthesis: An update. *Prostaglandins Leukotrienes and Essential Fatty Acids*, *85*(5), 205–213. https://doi.org/10.1016/j.plefa.2011.04.020

Brenner, M., Hearing, V. J. 2008. The protective role of melanin against UV damage in human skin. In *Photochemistry and Photobiology* 84(3):539–549. https://doi.org/10.1111/j.1751-1097.2007.00226.x

Browne, N., Heelan, M., Kavanagh, K. 2013. An analysis of the structural and functional similarities of insect hemocytes and mammalian phagocytes. *Virulence*, *4*(7), 597–603. https://doi.org/10.4161/viru.25906

Büyükgüzel, E., Tunaz, H., Stanley, D., Büyükgüzel, K. 2007. Eicosanoids mediate *Galleria mellonella* cellular immune response to viral infection. *Journal of Insect Physiology*, *53*(1), 99–105. https://doi.org/10.1016/j.jinsphys.2006.10.012

Carton, Y., Frey, F., Stanley, D. W., Vass, E., Nappi, A. J. 2002. Dexamethasone inhibition of the cellular immune response of *Drosophila melanogaster* against a parasitoid. *Journal of Parasitology*, *88*(2), 405–407. https://doi.org/10.1645/0022-3395(2002)088[0405:diotci]2.0.co;2

Chapman, R. F. 2013. *The İnsects* (S. J. SİMPSON and A. E. DOUGLAS, Eds.). Cambridge University Press.

Flajnik, M. F., Kasahara, M. 2010. Origin and evolution of the adaptive immune system: Genetic events and selective pressures. In *Nature Reviews Genetics*, 11(1), 47–59. https://doi.org/10.1038/nrg2703

Imler, J. L., & Zheng, L. 2004. Biology of Toll receptors: lessons from insects and mammals. Journal of Leucocyte Biology, 75(1), 18-26.

Kleino, A., Silverman, N. 2014. The *Drosophila* IMD pathway in the activation of the humoral immune response. *Developmental and Comparative Immunology*, *42*(1), 25–35. https://doi.org/10.1016/j.dci.2013.05.014

Merchant, D., Ertl, R. L., Rennard, S. I., Stanley, D. W., Miller, J. S. 2008. Eicosanoids mediate insect hemocyte migration. *Journal of Insect Physiology*, *54*(1), 215–221. https://doi.org/10.1016/j.jinsphys.2007.09.004

Miller, J. S. 2005. Eicosanoids influence in vitro elongation of plasmatocytes from the tobacco hornworm, *Manduca sexta*. *Archives of Insect Biochemistry and Physiology*, *59*(1), 42–51. https://doi.org/10.1002/arch.20052

Pereira, T. C., Barros, P. P. de, de Oliveira Fugisaki, L. R., Rossoni, R. D., Ribeiro, F. de C., Menezes, R. T. de, Junqueira, J. C., Scorzoni, L. 2018. Recent advances in the use of *Galleria mellonella* model to study immune responses against human pathogens. *Journal of Fungi*, *4*(4). https://doi.org/10.3390/jof4040128

Satyavathi, V. V., Minz, A., & Nagaraju, J. (2014). Nodulation: an unexplored cellular defense mechanism in insects. Cellular Signalling, 26(8), 1753-1763.

Schmucker, D., Clemens, J. C., Shu, H., Worby, C. A., Xiao, J., Muda, M., Dixon, J. E. 2000. Drosophila Dscam Is an Axon Guidance Receptor Exhibiting Extraordinary Molecular Diversity modified by neuronal activity (reviewed by Albright et al., 2000). Extraordinary progress has been made in identifying. In *Cell*, 101.

Sheehan, G., Garvey, A., Croke, M., Kavanagh, K. 2018. Innate humoral immune defences in mammals and insects: The same, with differences? *Virulence*, *9*(1), 1625–1639. https://doi.org/10.1080/21505594.2018.1526531

Shrestha, S., Kim, Y. 2008. Eicosanoids mediate prophenoloxidase release from oenocytoids in the beet armyworm *Spodoptera exigua*. *Insect Biochemistry and Molecular Biology*, *38*(1), 99–112. https://doi.org/10.1016/j.ibmb.2007.09.013

Silverman, N., Maniatis, T. 2001. NF-κB signaling pathways in mammalian and insect innate immunity. *Genes and Development*, *15*(18), 2321–2342. https://doi.org/10.1101/gad.909001

Stanley, D. W., Miller, J. S. 2006. Eicosanoid actions in insect cellular immune functions. In *Entomologia Experimentalis et Applicata*, 119(1), 1–13. https://doi.org/10.1111/j.1570-7458.2006.00406.

Stanley, D., & Kim, Y. (2019). Insect prostaglandins and other eicosanoids: From molecular to physiological actions. In *Advances in Insect Physiology* (Vol. 56, pp. 283-343). Academic Press.

Stanley, D., Miller, J., Tunaz, H. 2009. Eicosanoid actions in insect immunity. In *Journal of Innate Immunity*, 1(4), 282–290. https://doi.org/10.1159/000210371

Stanley-Samuelson, D. W., Jensent, E., Nickersonf, K. W., Tiebelt, K., Ogg, C. L., Howard, R. W. 1991. Insect immune response to bacterial infection is mediated by eicosanoids (cyclooxygenase/lipoxygenase/phospholipase A2/*Manduca sexta*/*Serratia marcescens).* In *Physiology*, 88, 1064-1068.

Tunaz, H. 2004. Böceklerde Bağışıklık Mekanizması. *KSU. Journal of Science and Engineering*, *7*(2), 78–82.

Tunaz, H., Işıkber, A. A., Er, M. K. 2018. Alman Hamamböceği *Blatella germanica* (L.)'nın Erginlerinde İki Fungal İzolata Karşı Eikosanoidlerin Nodülasyon Reaksiyonları Üzerine Olan Fonksiyonu. *KSU J. Agric Nat*, *21*(3), 428–432. https://doi.org/10.18016/ksudobil

Zhang, W., Tettamanti, G., Bassal, T., Heryanto, C., Eleftherianos, I., Mohamed, A. 2021. Regulators and signalling in insect antimicrobial innate immunity: Functional molecules and cellular pathways. *Cellular Signalling*, *83*(110003). https://doi.org/10.1016/j.cellsig.2021.110003

IN VITRO EVALUATION OF THE ANTIFUNGAL ACTIVITY OF *LACTOBCILLUS* STRAINS AGAINST PHYTOPATHOGENIC FUNGI

Meglali Amina[(1)*], Adli Djallal Eddine[(1)], Amara Sabrina[(2)], Benreguieg Mokhtar[(3)]

1. Laboratory of Bio-toxicology, Pharmacognosy and Biological Valorization of Plants (LBPBVP), University of Saida Dr Moulay Tahar. *20000 Saida, Algeria ;*
2. *Laboratory of Biology of Micro-organisms and Biotechnology, University of Oran 1, PB 1524, El M'Naouer, Oran 31000, Algeria;*
3. Department of Biology, Faculty of sciences, University of Saida Dr Moulay Tahar, Ennasr 20000, Saida, Algeria.

Corresponding author: meglaliamina@gmail.com

ABSTRACT

Lactic acid bacteria include several genera, the majority of which are represented by *Lactobacillus*. They are currently the focus of a great deal of research due to their potential in various biotechnological applications. They have demonstrated their ability to produce antimicrobial agents such as lactic acid, hydrogen peroxide, and bacteriocins, which may act synergistically against filamentous fungi.

These properties of *Lactobacillus*, particularly their antimicrobial potential, raise important questions linked to the production of antifungal agents, bio-preservation and the fight against fungal deterioration. These issues are of fundamental importance in diverse fields such as agriculture, biotechnology, food industry and more.

In our experiment, we have demonstrated the antifungal activity of four lactic acid bacteria isolated from camel and goat milk, designated L1, L2, L3, and L4, against four phytopathogenic fungal strains (*Aspergillus niger*; *Phytophtora infestans*; *Penicilium spp*; et *Endosporium spp*). This evaluation was carried out using different levels of concentrations of the bacterial supernatant, namely 0.25, 1, 1.75, and 2.5 µl/ml.

Our strains showed strong antifungal activity, even at low concentrations, with a significantly different effect from one strain to another. All four supernatants had a significant effect against *Penicillium spp*, *A.niger*, and *P.infestans*, with an antifungal index between [43.33 - 63] %, [38.46 - 61.54] %, and [35.56 - 55.56] % respectively. Furthermore, the best antifungal activity against *Endosporium spp* was obtained by strains L4 and L1 with inhibitory effects equal to 38.24% and 26.47%.

These results provide a clear idea of the antimicrobial potential of *Lactobacillus* strains, which represent an alternative to some antifungal agents used in different fields (agriculture, biotechnology, …etc).

Keywords: *Lactobacillus*, filamentous fungi, antifungal activity, agriculture, biotechnology.

INTRODUCTION

Lactic acid bacteria (LAB) are a diverse group of microorganisms, predominantly represented by the genus Lactobacillus, that have garnered significant attention in recent years for their versatile applications in biotechnology. These bacteria are known for their ability to produce various antimicrobial substances, including lactic acid, hydrogen peroxide, and bacteriocins, which can work synergistically to inhibit the growth of filamentous fungi. The antimicrobial properties of Lactobacillus not only highlight their potential as natural preservatives but also raise critical questions regarding their role in antifungal agent production and bio-preservation strategies across multiple sectors such as agriculture and the food industry.

In this study, we explore the antifungal capabilities of four specific Lactobacillus strains isolated from camel and goat milk, designated L1, L2, L3, and L4. These strains were tested against four phytopathogenic fungal species: *Aspergillus niger*, *Phytophthora infestans*, *Penicillium spp.*, and *Endosporium spp.* The experimental design included varying concentrations of bacterial supernatants to assess their efficacy in inhibiting fungal growth. The results demonstrated notable antifungal activity across all strains, particularly against *Penicillium spp.*, *A. niger*, and *P. infestans*, suggesting that these LAB could serve as effective biocontrol agents in combating fungal deterioration in various applications. This research underscores the potential for Lactobacillus strains to offer sustainable alternatives to synthetic antifungal agents in diverse fields.

Materials and Methods

Strains Used:

- Four *Lactobacillus* strains (L1, L2, L3, L4) were selected from the collection at the Laboratory of Biology of Microorganisms and Biotechnology (LBMB), University of Oran.

Strain Revivification:

- The bacterial strains were revivified in skim milk and MRS broth at 30°C, **Figure 1.**

Figure 4: Revival of lactic strains.

Fungal Strains Tested:

- Four phytopathogenic fungal species were studied: *Aspergillus niger*, *Phytophthora infestans*, *Penicillium spp.*, and *Endosporium spp*. The purification of fungal species was carried out by a series of subcultures, which consists of transferring a part of the mycelium of each colony into a PDA box and then incubating it for 3-5 days at 28°C. The identification used is based on the combination of macroscopic and microscopic observations **(Engelkirk et al., 2011):**
- • Macroscopic observation is based on the cultural characteristics observed with the naked eye.
- • Microscopic observation is based on the morphological characteristics of mycelium and spores **(Griensven, 2000),** and was evaluated by the adhesive tape technique **(chabasse et *al*., 1999)** which consists of pressing on the surface of the colony using a small piece of transparent tape, then stuck to a glass slide covered with lactophenol cotton blue and observed under a microscope.

Antifungal Activity Testing:

- The antifungal activity of the Lactobacillus strains was assessed by introducing their supernatants into melted PDA medium at 45°C (**Soliman et Badeaa, 2002**), **Figure 2.**
- Different concentrations of the supernatants (100, 400, 700, 1000 µl) were tested.
- The plates were incubated at 28°C for five days.
- The antifungal activity was calculated using the formula: I = {Dt - Da}/{Dt}×100

Where I is the inhibition index, Dt is the diameter of the control colony, and Da is the diameter of the colony in the presence of the bacterial supernatant.

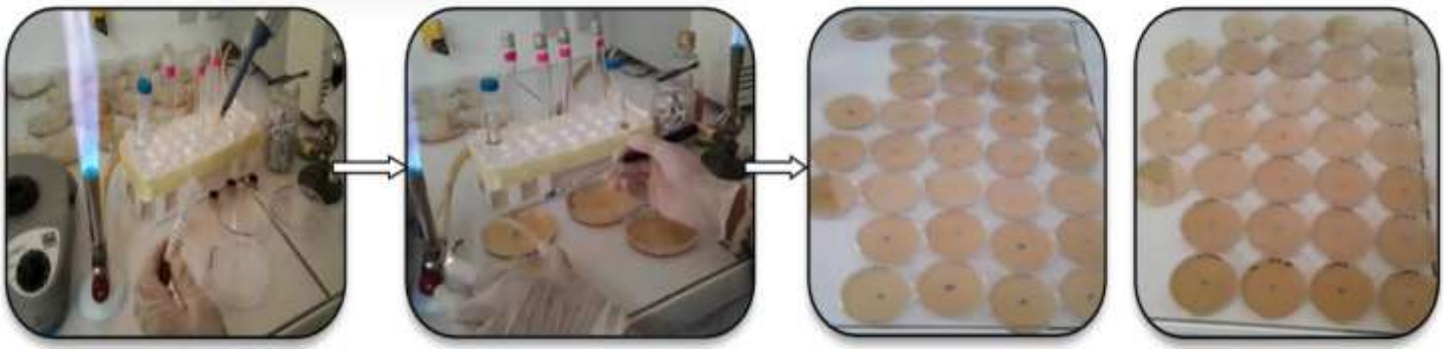
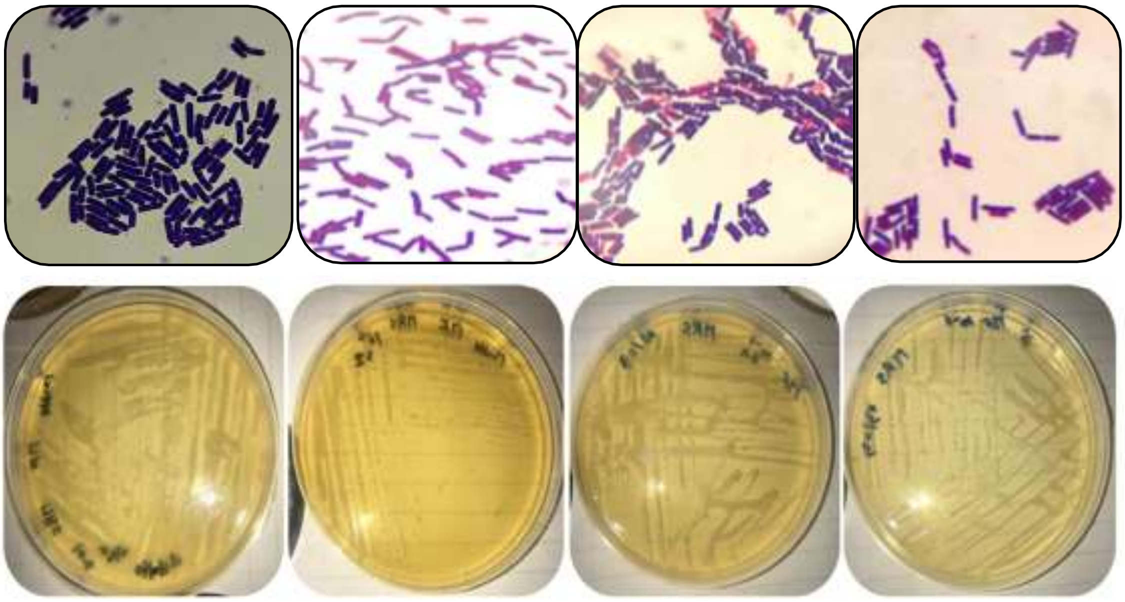


Figure 5: The realization of antifungal activity.

RESULTS AND DISCUSSION

Revivification and identification

Two types of observation were necessary to determine the purity of the bacterial cultures after subculturing. Macroscopic observation revealed that the colonies of the different strains have the same phenotypic characteristics, elongated shape with regular outline, whitish color and creamy appearance **(Figure 3).** Microscopic observation after Gram staining showed the shape and purity of the cells: the bacteria are Gram-positive rods, isolated or in pairs. Among the four strains examined, none of them showed catalase activity.



L1 L2 L3 L4

Figure 6: Macroscopic and microscopic appearance of lactic strains

Antifungal Activity

The results showed that the supernatant of the four Lactobacillus strains (SL1, SL2, SL3 and SL4) exhibited more or less significant antifungal activity depending on the strain and the different concentrations of the supernatant, **Table 2.**

Table 1: Effect of supernatants on the growth diameter of fungal strains.

Strains	Fungal strains	Growth diameter (mm) with different concentrations of supernatant (µl)				
		T	100	400	700	1000
SL1	*Penicillium spp*	30	18	17	16	14
	Aspergillus niger	39	30	30	19	15
	Phytophtora infestans	45	38	33	30	20
	Endosporium spp	34	31	30	29	25
SL2	*Penicillium spp*	30	21	16	14	11
	Aspergillus niger	39	31	30	25	23
	Phytophtora infestans	45	34	33	29	29
	Endosporium spp	34	32	30	30	28
SL3	*Penicillium spp*	30	22	20	19	16
	Aspergillus niger	39	35	30	25	24
	Phytophtora infestans	45	35	33	33	20
	Endosporium spp	34	34	32	32	30
SL4	*Penicillium spp*	30	22	20	19	17
	Aspergillus niger	39	33	30	30	22
	Phytophtora infestans	45	33	29	28	28
	Endosporium spp	34	29	25	24	21

All concentrations of SL1 supernatant cause a significant effect on *Aspergillus niger* and Penicillium spp by an antifungal index equal to 61.54% (50µl/ml) and 53.33% (50µl/ml) respectively. The antifungal index equal to 55.56% of the 50µl/ml concentration and 33.33% at 35µl/ml is considered the highest and rather significant for *Phytophtora infestans*. The lowest index is measured on *Endosporium spp* in the high concentrations: 26.47% (50µl/ml) and 14.71% (35µl/ml), **Figure** 4.

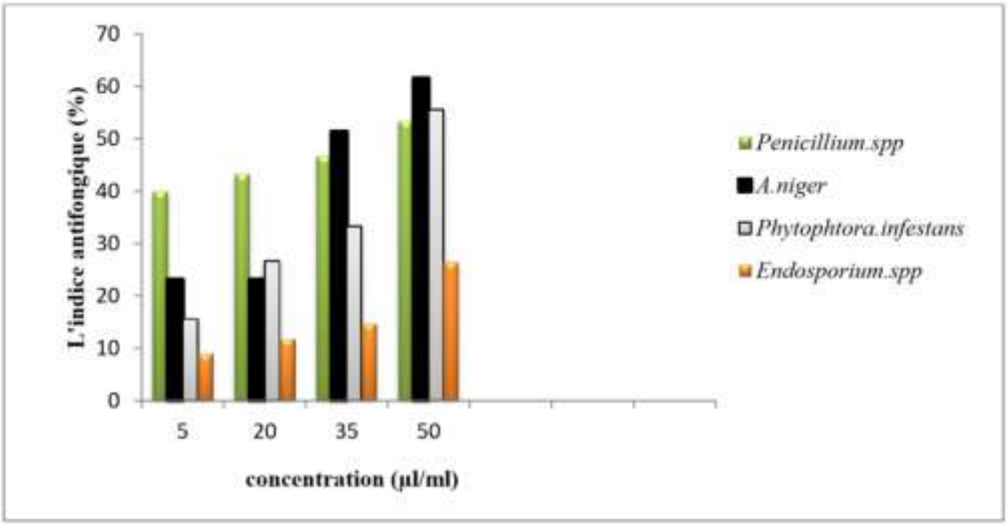


Figure 7: Antifungal effect of SL1 supernatant.

The best activity of the supernatant from the Lactobacillus 2 strain was obtained against *Penicillium.spp*, with an inhibition rate exceeding 63% at 50µl/ml and 53% at 35µl/ml. *Aspergillus niger* and *Phytophthora infestans* respectively give an antifungal index equal to 38.46% (50µl/ml) and 35.56% (50 and 35µl/ml). The supernatant of *Lactobacillus* 2 is weakly influential with high concentrations especially against *Endosporium spp* with an antifungal index equal to 11.76% (20 and 35µl/ml) and 17.65% (50µl/ml), **Figure 5**.

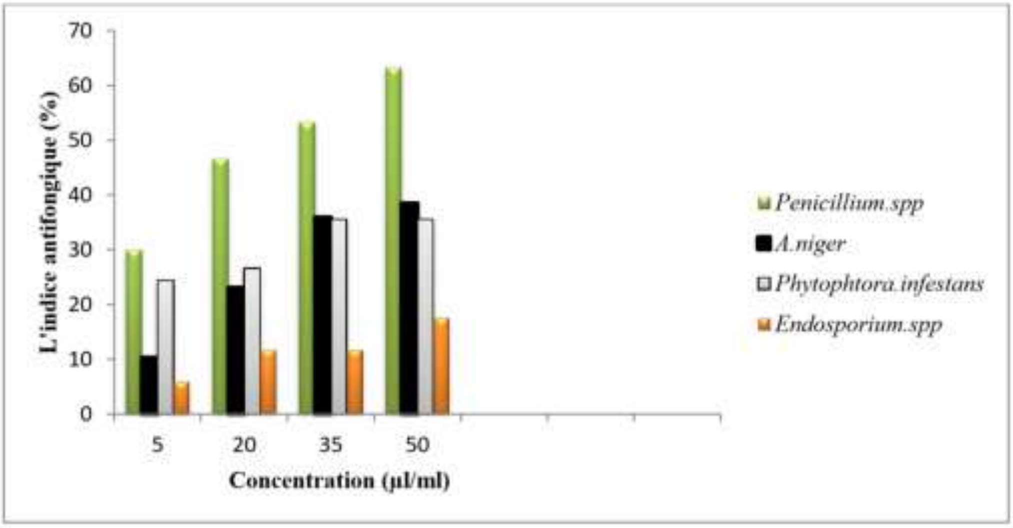


Figure 8: Antifungal effect of SL2 supernatant.

The analysis of the results illustrated in **Figure 6** allowed us to note that *Phytophthora infestans* is the most sensitive strain with respect to the SL3 supernatant with an inhibition rate equal to 55.56% (50µl/ml) and 26.67% (35µl/ml). The antifungal index in high concentrations (50µl/ml) reaches 46.67% for *Penicillium spp,* 38.46% for *Aspergillus niger* and greater than 35% for concentrations of 35µl/ml. No antifungal activity was detected on *Endosporium.spp* for low concentrations 5 and 20µl/ml.

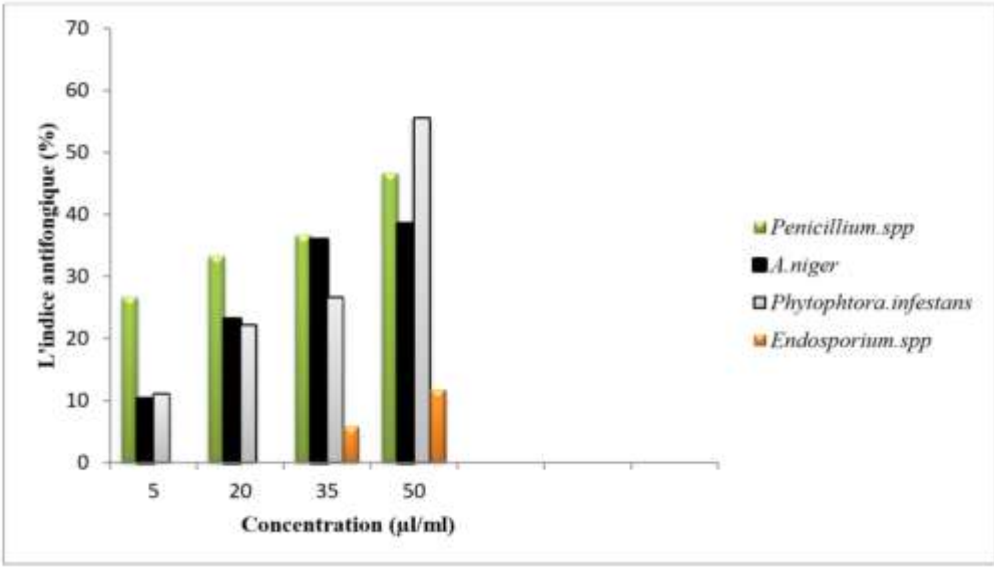


Figure 9: Antifungal effect of SL3 supernatant.

The observation of the histogram of **Figure** 7 relating to the antifungal effect of the supernatant SL4, shows that all the concentrations of the latter cause a significant effect on the four fungal strains with an antifungal index between 37.78% and 43.59% for the concentrations of 50µl/ml and between 23.08% and 37.78% for the concentrations of 35µl/ml. The antifungal index in the low concentrations is higher than 33% (20µl/ml) for *Penicillium spp* and *Phytophtora infestans* and higher than 23% for *Aspergillus niger* and *Endosporium spp.*

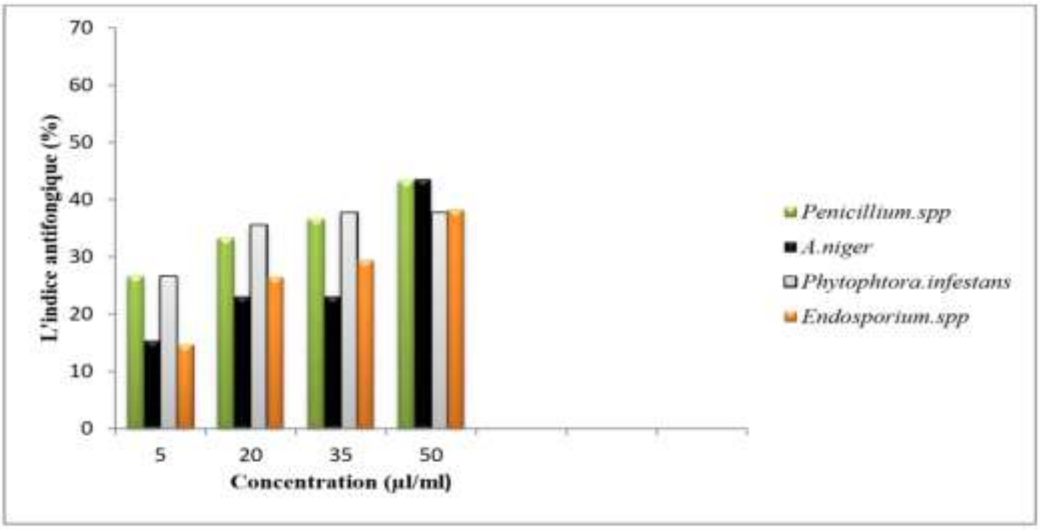


Figure 10: Antifungal effect of SL4 supernatant.

Lactic acid bacteria produce a wide range of compounds that could act synergistically against yeasts and filamentous fungi **(Magnusson et *al*., 2003). Cabo et *al* (2002)** suggested that the antifungal activity of lactic acid bacteria is due to a synergistic effect between lactic acid produced by the bacteria and acetic acid from the culture medium. Many studies have shown that lactic acid bacteria produce antifungal compounds that are different from organic acids, these compounds are difficult to isolate and characterize because of their low concentrations, chemical composition, being synergistically active molecules with low individual activity, or other unknown reasons **(Nes et *al*., 2011).** The majority of purified antifungal substances have been low molecular weight compounds such as: phenylacetic acid, cyclic dipeptides and short or medium chain fatty acids **(Schnürer and Magnusson, 2005).**

CONCLUSION

The results of this study demonstrate the significant antifungal potential of the four *Lactobacillus* strains (L1, L2, L3, L4) tested against common phytopathogenic fungi, including *Aspergillus niger*, *Phytophthora infestans, Penicillium spp,* and *Endosporium* spp. All strains exhibited notable antifungal activity, with varying degrees of effectiveness depending on the fungal species and the concentration of bacterial supernatants. The strains L1 and L4 showed the highest inhibition against *Endosporium spp*, indicating their strong antifungal properties. These findings suggest that *Lactobacillus* strains can serve as a promising alternative to conventional antifungal agents, offering a natural and effective solution for controlling fungal pathogens. This work highlights the potential for utilizing lactic acid bacteria in agricultural, biotechnological, and food preservation applications, contributing to the reduction of chemical fungicide use and combating fungal resistance.

REFERENCES

Cabo M L., Braber A F., Koenraad P M F J. 2002. « Apparent antifungal activity of several lactic acid bacteria against Penicillium discolor is due to acetic acid in the medium ». Journal of Food Protection. 65(8):1309-1316.

Chabasse D., Guiguen C., Contet-Audonneau N. 1999. « Mycologie médicale ». Collection abrégé.

Engelkirk P. G., Engelkirk-Duben L. J., Burton G. R. W. 2011. « Burton microbiology for the health ».

Nes I F., Kjos M., Diep D B. 2011. «Antimicrobial components of lactic acid bacteria». In: Lactic Acid Bacteria: Microbiological and Functional Aspects. 4th ed. CRC Press, London. 285-329.

Schnürer J et Magnusson J. 2005. «Antifungal lactic acid bacteria as biopreservatives». Trends in Food Science & Technology. 16 (1): 70-78.

Sharma A, Lavania M, Singh R, Lal B. 2020. Identification and probiotic potential of lactic acid bacteria from camel milk. Saudi J Biol Sci. 2021 Mar;28(3):1622-1632.

Soliman K.M et Badeaa R.I. 2002. « Effect of oil extracted from some medicinal plants on different mycotoxigenic Fungi ». Food and Chemical Toxicology; 4: 1669–1675.

RESISTANCE TO QUATERNARY AMMONIUM COMPOUNDS of *Staphylococcus aureus* ISOLATED FROM CHICKEN MEAT

Fatma Özdemir[1], Seza Arslan[1]

[1] *Bolu Abant Izzet Baysal University, Faculty of Arts and Sciences, Department of Biology, 14030 Gölköy / Bolu, Türkiye*
Corresponding author e-mail: ozkardes_f@ibu.edu.tr

ABSTRACT

Staphylococcus aureus is an important pathogen due to its serious human and animal health problems worldwide. It causes a wide variety of diseases, including skin and soft tissue infections, mastitis, osteomyelitis, bacteraemia, endocarditis, pneumonia, foodborne intoxication, and hospital- and community-acquired infections. Many disinfectants, including quaternary ammonium compounds (QACs) as active biocides, have been widely used for sanitization in the food industry, poultry processing facilities, veterinary medicine, hospitals and healthcare facilities, and environments that could promote the development of bacterial resistance to disinfectants. The aim of the present study was to investigate the susceptibility of *S. aureus* isolated from chicken meat to quaternary ammonium compound (QAC) disinfectants, including benzalkonium chloride (BKC) and cetylpyridinium chloride (CPC). The minimum inhibitory concentration (MIC) of these QACs against the chicken meat isolates of *S. aureus* was determined quantitatively using a broth microdilution method. The MIC values of BKC were ranged between 1 and 8 µg/ mL, while the MICs for CPC were ranged between 0.5 and 2 µg/ mL. Of the isolates, 53.8% were found to be resistant to BKC with MICs of over 2 µg/ mL. The results of this study demonstrated the antimicrobial efficacy of quaternary ammonium compounds BKC and CPC on *S. aureus* isolated from chicken meat, which could be useful for food processing and consumer health in preventing and controlling foodborne pathogens, including resistant bacteria.

Keywords: Quaternary ammonium compounds, Chicken meat, *Staphylococcus aureus*, Minimum inhibitory concentration, Broth microdilution method

INTRODUCTION

Staphylococcus aureus is a ubiquitous Gram-positive, nonmotile, and facultatively anaerobic bacterial pathogen associated with hospital- and community-acquired infections. It causes a wide variety of human diseases including skin and soft tissue infections, food poisoning, bacteremia, toxic shock syndrome, pneumonia and endocarditis (Götz et al., 2006; Bhunia, 2008; Bannerman and Peacock, 2011). *S. aureus* is often found on the skin, nares and mucous membranes of human and warm-blooded animals including all food animals (Götz et al., 2006; Bannerman and Peacock, 2011). *S. aureus* contamination is associated with foods including raw milk, milk products, puddings, pork sausage, ham, meats, fish, shellfish, salads and vegetables (Bhunia, 2008; Beier et al., 2021). Moreover, cross-contamination in food production chain can occur by food handlers or preparers, equipment, and contaminated food contact surfaces (Bhunia, 2008).

Antimicrobial resistance is the most serious clinical and public health problem around the world. The overuse or misuse of antimicrobial agents in humans, animals and plants has dramatically accelerated the emergence and spread of antimicrobial-resistant strains including methicillin-resistant *Staphylococcus aureus* (MRSA) (Götz et al., 2006; WHO, 2023). MRSA strains, as a serious and enduring threat to human health, are typically resistant to all beta-lactam antimicrobial agents, which are commonly used to treat infections (David and Daum, 2010; Bannerman and Peacock, 2011). Thus, the treatment of infections caused by these antimicrobial-resistant pathogens may be more difficult and expensive than infections caused by methicillin-susceptible *S. aureus* (MSSA) (David and Daum, 2010).

Quaternary ammonium compounds (QACs) have been used as common disinfectants in controlling bacterial growth in hospitals, healthcare settings, food industries, veterinary practices, and environments due to their broad-spectrum antimicrobial activities and surfactant properties (McDonnell and Russel, 1999; Bjorland et al., 2001; McBain et al., 2004; Wu et al., 2015). The antimicrobial activity of QACs corresponds with the side chain's hydrophobicity and demonstrates a maximum impact when the alkyl chain has 12 -16 carbon atoms. QACs inhibit spore growth but not the germination processes due to their sporostatic properties, (Marris, 1995; McDonnell and Russel, 1999; Mao et al., 2020). Among the QACs, benzalkonium chloride (BKC) and cetylpyridinium chloride (CPC) are two of the most frequently used QACs. BKC is a cationic surfactant with a broad-spectrum antimicrobial activity except for bacterial endospores. Its bactericidal effectiveness has been linked to a change in the permeability of the cytoplasmic membrane after the lipid bilayers in the cell membrane dissociate, allowing cell contents to leak out (Leggett et al., 2016; Maillard et al., 2022). CPC, a monocationic surfactant, has both hydrophilic and lipophilic properties derived from the chloride and cetylpyridinium cations, respectively (Lim et al., 2004).

The objective of this research was to investigate the antibacterial efficacy of quaternary ammonium compounds, including benzalkonium chloride and cetylpyridinium chloride, against the foodborne pathogen *S. aureus* using the broth microdilution method.

MATERIALS AND METHODS

Bacterial Isolates

In this study, thirteen *S. aureus* isolates from samples of chicken meat consisting of breast and leg parts identified in previous research (Özdemir, 2022) were tested. All isolates were identified using biochemical tests and PCR assays for the *S. aureus*-specific fragment (Sa442) and thermonuclease gene (*nucA*) (Brakstad et al., 1992; Martineau et al., 1998; Götz et al., 2006; Bannerman and Peacock, 2011). The isolates were identified in our earlier study (Özdemir, 2022) as methicillin-resistant *S. aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA) utilizing the *mecA* gene (Milheiriço et al., 2007). Before the experiments were done, the isolates were cultured overnight in Brain Heart Infusion broth (BHI) (Merck, Darmstadt, Germany).

Quaternary ammonium compounds

Quaternary ammonium compounds (QACs) used in the present research included benzalkonium chloride (BKC) and cetylpyridinium chloride (CPC). These compounds were purchased from Sigma-Aldrich (MO, USA). Stock solutions of BKC and CPC were prepared and sterilized through a 0.45 µm membrane syringe filter (Sartorius AG, Goettingen, Germany). The tested concentrations for BKC ranged from 0.25 to 128 µg/mL and for CPC ranged from 0.5 to 256 µg/mL.

Examination of antimicrobial efficacy of quaternary ammonium compounds

The minimum inhibitory concentrations (MIC) of quaternary ammonium compounds (QACs) including benzalkonium chloride (BKC) and cetylpyridinium chloride (CPC) against the *S. aureus* isolates from chicken meat were determined using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, 2012; CLSI, 2022). Bacterial suspensions were prepared, and the turbidity was adjusted to the 0.5 McFarland standard. The suspensions were then diluted 1:100 in Mueller-Hinton (MH) broth to obtain final inoculum suspensions. The sterile U-bottom 96-well microtiter plates (Lp Italiana) were used. The microtiter plates were filled with MH broth with a two-fold concentration of QACs solution and 50 µl of the bacterial suspension (adjusted final inoculum density of 10^6 CFU/mL). *Staphylococcus aureus* ATCC 29213 was used as a quality control strain to determine MIC values in all experiments. The 96-well microtiter plates were incubated for 16-20 h at 37 ºC. The optical density (OD) was measured using a microplate reader (Thermo Electron Corporation, Vantaa, Finland) at 600 nm. The MICs of the isolates against the QACs were detected as the lowest concentration with no visible growth (Andrews, 2001; CLSI, 2012). The *S. aureus* isolates were categorized as resistant to BKC at MICs > 2 µg/mL and to CPC at MICs > 1 µg/mL. based on the comparison with the control strain (*S. aureus* ATCC 29213).

RESULTS AND DISCUSSION

Quaternary ammonium compounds (QACs) are known as cationic detergents with broad-spectrum antimicrobial properties (McDonnell and Russell, 1999; McBain et al., 2004). QACs such as cetylpyridinium chloride (CPC) and benzalkonium chloride (BKC), have been shown to have antimicrobial properties against a variety of pathogens, including foodborne pathogens, in earlier investigations (Lim et al., 2004; Wu et al., 2015; Mao et al., 2020; Beier et al., 2021). Table 1 summarized the minimum inhibitory concentrations (MICs) of BKC and CPC against the isolates of *S. aureus* from chicken meat.

Table 1. The minimum inhibitory concentration (MIC) of benzalkonium chloride (BKC) and cetylpyridinium chloride (CPC) against the *S. aureus* isolates from chicken meats

Disinfectants	Resistance (%)	Percentages of the isolates with MIC values (μg/ml)										
		256	128	64	32	16	8	4	2	1	0.5	0.25
BKC	53.8						**7.7**	**46.1**	30.8	15.4		
CPC	15.4								**15.4**	23.1	61.5	

The resistance levels of the isolates were determined by comparing their minimum inhibitory concentration (MIC) values to those of *S. aureus* ATCC 29213. *The MIC value of the isolate > 2 µg/mL for benzalkonium chloride (BKC) and > 1 µg/mL for cetylpyridinium chloride (CPC) was accepted as a resistant isolate. The percentages of the resistant isolates are displayed in bold font.*

Of the S. aureus *isolates used in this study, 53.8% showed BKC resistance (MIC > 2 µg/mL) and 15.4% exhibited CPC resistance (MIC > 1 µg/mL).* S. aureus *isolates (46.15%) had susceptible BKC MICs that ranged from 1-2 µg/mL.* Furthermore, it was determined that 84% of the isolates were susceptible to CPC, which had a range of 0.5 to 1 µg/mL (Table 1). Wu et al. (2015) noticed that the MICs varied based on the type of QACs and the species of bacteria. QACs, such as cetylpyridinium chloride and benzalkonium chloride, have antibacterial activity against foodborne and zoonotic pathogenic bacteria. They observed that Gram-positive strains, including *S. aureus* were extremely susceptible to QACs. All *S. aureus* strains isolated from swine feces were susceptible to *benzalkonium chloride* (at MICs < 30 µg/mL) and cetylpyridinium chloride. MIC values ranged from 0.5-1µg/mL for *benzalkonium chloride* and ranged from 0.0625-1µg/mL for cetylpyridinium chloride (Beier et al., 2021).

The determined MIC values of benzalkonium chloride and cetylpyridinium chloride (CPC) against the isolates, including methicillin-sensitive S. aureus *(MSSA) and methicillin-resistant* S. aureus *(MRSA) isolates, were represented in Figures 1 and 2. The MIC values of benzalkonium chloride (BKC) against the MSSA isolates (66.7%) ranged between 0.25 and 2 µg/mL. Furthermore, 33.3% of the MSSA isolates for BKC showed the MIC at higher concentrations (≥4 µg/mL) and were recorded as resistant. Remarkably, all MRSA isolates were found to be resistant to BKC (Figure 1).*

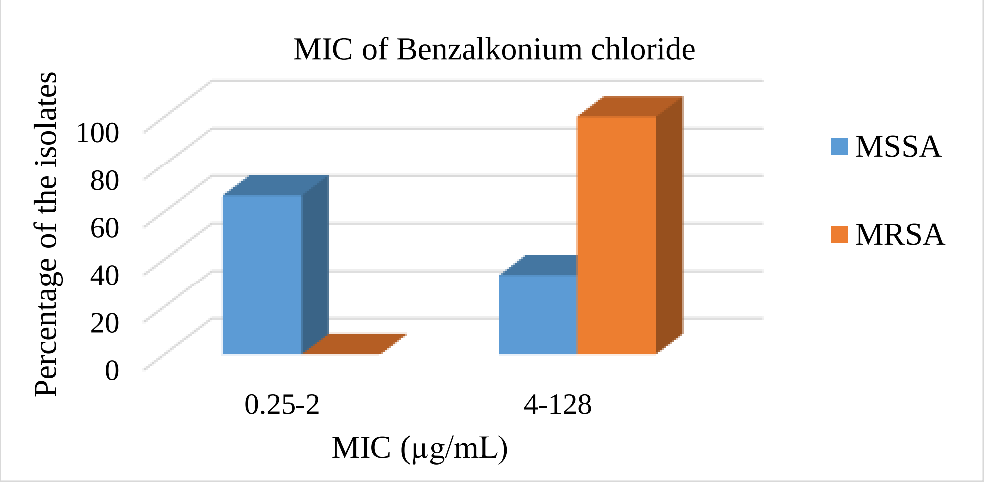


Figure 1. Distribution of MIC values of benzalkonium chloride in the MSSA and MRSA isolates. MSSA: Methicillin-sensitive S. aureus, *MRSA: methicillin-resistant* S. aureus.

Figure 2 demonstrates the MIC distribution of the cetylpyridinium chloride (CPC) in the MSSA and MRSA isolates. Of the MSSA isolates, 11.1% had MIC values of 2 µg/mL for cetylpyridinium chloride (CPC), whereas 88.9% exhibited MIC ranges of 0.5 and 1 µg/mL for CPC. Moreover, a MIC range between 0.5 and 1 µg/mL was found in 75% of the MRSA isolates, while 2 µg/mL was found in 25% of the MRSA isolates.

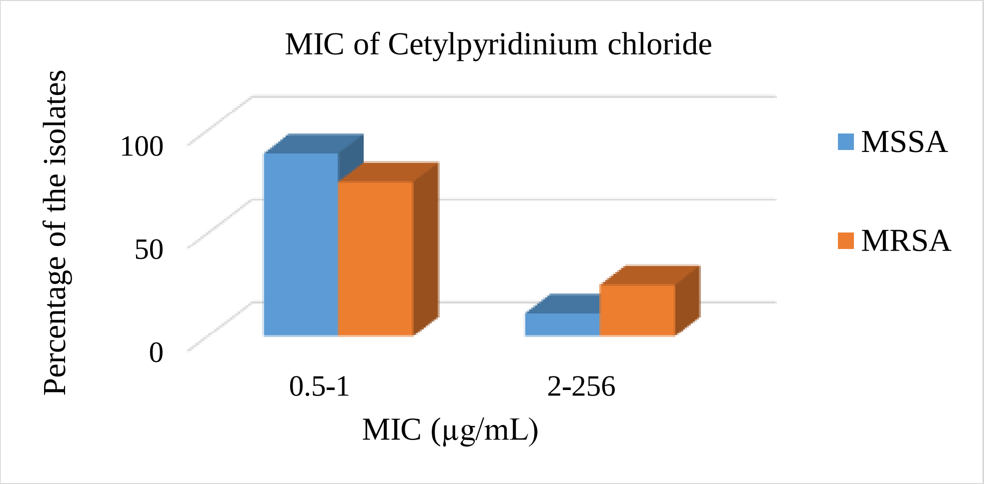


Figure 2. Distribution of MIC values of cetylpyridinium chloride *in the MSSA and MRSA isolates. MSSA: Methicillin-sensitive* S. aureus, *MRSA: methicillin-resistant* S. aureus.

Our results indicated that higher MIC values for the tested quaternary ammonium compounds were determined in MRSA isolates compared with MSSA isolates. As compared to MSSA strains, MRSA strains were found to be highly resistant not only to oxacillin and but also *benzalkonium chloride with MIC values* of *10 µg/ml in a previous study* (Akimitsu et al.,1999). *In contrast*, six MRSA strains displayed increased susceptibilities to disinfectants, including QACs, by Beier et al. (2021). Suller et al. (1999) investigated the susceptibilities of MRSA and MSSA strains to cetylpyridinium chloride. *They demonstrated that MICs of 2 or 4 µg/ml for MRSA were found to exhibit "low-level resistance" to cetylpyridinium chloride, but the MSSA strain had MICs of 1 µg/ml.*

CONCLUSION

This study showed the antibacterial activity of quaternary ammonium compounds (QACs), including benzalkonium chloride and cetylpyridinium chloride. They are widely used as disinfectants and sanitizers in variety of applications including food industry against the *S. aureus* isolates originated from chicken meat. The antimicrobial activity of every QAC varies according to their chemical properties and the type of bacteria.

REFERENCES

Andrews, J.M. 2001. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 48, 5-16.

Akimitsu, N., Hamamoto, H., Inoue, R., Shoji, M., Akamine, A., Takemori, K., Hamasaki, N., Sekimizu, K. 1999. Increase in resistance of methicillin-resistant *Staphylococcus aureus* to β-lactams caused by mutations conferring resistance to benzalkonium chloride, a disinfectant widely used in hospitals. Antimicrobial Agents and Chemotherapy, 43(12), 3042-3043.

Bannerman, T.L., Peacock, S.J. 2011. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, eds. Manual of clinical microbiology, Washington, D.C: ASM Press, 308-330.

Beier, R.C., Andrews, K., Hume, M.E., Sohail, M.U., Harvey, R.B., Poole, T.L. Crippen, T.L., Anderson, R.C. Robin C. 2021. Disinfectant and antimicrobial susceptibility studies of Staphylococcus aureus strains and ST398-MRSA and ST5-MRSA strains from swine mandibular lymph node tissue, commercial pork sausage meat and swine feces. Microorganisms, 9, 2401.

Bhunia, A.K. 2008. Foodborne microbial pathogens: Mechanisms and pathogenesis. New York, N.Y.: Springer.

Bjorland, J., Sunde, M., Waage, S. 2001. Plasmid-borne smr gene causes resistance to quaternary ammonium compounds in bovine *Staphylococcus aureus*. Journal of Clinical Microbiology, 39, 3999-4004.

Brakstad, O.G., Aasbakk, K., Maeland, J.A. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. Journal of Clinical Microbiology, 30, 1654-1660.

Clinical and Laboratory Standards Institute (CLSI). 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Ninth edition, M7-A9, in, Wayne, PA, USA.

Clinical and Laboratory Standards Institute (CLSI). 2022. Performance standards for antimicrobial susceptibility testing; 32nd edition, in, Malvern, PA, USA.

David, M., Daum, R.S. 2010. Community-associated methicillin-resistant *Staphylococcus aureus*: Epidemiology and clinical consequences of an emerging epidemic. Clinical Microbiological Reviews, 23 (3), 616-687.

Götz, F., Bannerman, T., Schleifer, K.H. 2006. The genera *Staphylococcus* and *Macrococcus*. In: The Prokaryotes. Editors: M. Dworkin, S. Falkow, E. Rosenberg, K.H. Schleifer, E. Stackebrandt, Springer: USA. 5-75.

Leggett, M., Setlow, P., Sattar, S.A., Maillard, J.-Y. 2016. Assessing the activity of microbicides against bacterial spores: knowledge and pitfalls. Journal of Applied Microbiology, 120, 1174-1180.

Lim, K., Mustapha, A. 2004. Effects of Cetylpyridinium Chloride, Acidified Sodium Chlorite, and Potassium Sorbate on Populations of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Staphylococcus aureus* on Fresh Beef. Journal of Food Protection, 67(2), 310-315.

Maillard, J-Y. 2022. Impact of benzalkonium chloride, benzethonium chloride and chloroxylenol on bacterial antimicrobial resistance. Journal of Applied Microbiology, 133, 3322-3346.

Mao, X., Auer, D.L., Buchalla, W., Hiller, K-A., Maisch, T., Hellwig, E., Al-Ahmad, A., Cieplik, F. 2020. Cetylpyridinium chloride: mechanism of action, antimicrobial efficacy in biofilms, and potential risks of resistance. Antimicrobial Agents and Chemotherapy, 64(8), e00576-20. https://doi.org/10 .1128/AAC.00576-20

Maris, P. 1995. Modes of action of disinfectants. Revue scientifique et technique, 14, 47-55. https://doi.org/10.20506/rst.14.1.829

Martineau, F., Picard, F.J., Roy, P.H., Quellette, M., Bergeron, M.G. 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. Journal of Clinical Microbiology, 36, 618-623.

McBain, A.J., Ledder, R.G., Moore, L.E., Catrenich, C.E., Gilbert, P. 2004. Effects of quaternary-ammonium–based formulations on bacterial community dynamics and antimicrobial susceptibility. Applied and Environmental Microbiology, 70(6), 3449-3456.

McDonnell, G., Russell, A.D. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clinical Microbiological Reviews, 12, 147-179.

Milheiriço, C., Oliveira, D.C., De Lencastre, H. 2007. Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy, 51, 3374-3377.

Özdemir, F. 2022. Antimicrobial Resistance, Multilocus Sequence, and spa Typing of *Staphylococcus aureus* Isolated from Retail Raw Meat Products. BioMed Research International, 6035987.

Suller, M.T.E., Russell, A.D. 1999. Antibiotic and biocide resistance in methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococcus. Journal of Hospital Infection, 43, 281-291.

World Health Organization (WHO). 2023. Antimicrobial resistance. 21 November 2023. Available online: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Wu, G., Yang, Q., Long, M., Guo, L., Li, B., Meng, Y., Zhang, A., Wang, H., Liu, S., Zou, L. 2015. Evaluation of agar dilution and broth microdilution methods to determine the disinfectant susceptibility. The Journal of Antibiotics, 68, 661-665.

ANTIBACTERIAL ACTIVITY OF GOLD NANOPARTICLES AGAINST *Staphylococcus aureus*

Seza Arslan[1], *Fatma Özdemir*[1]

[1] *Bolu Abant Izzet Baysal University, Faculty of Arts and Sciences, Department of Biology, 14030 Gölköy / Bolu, Türkiye*
Corresponding author e-mail: ozkardes_f@ibu.edu.tr

ABSTRACT

Infections caused by antimicrobial-resistant and multidrug-resistant bacteria such as *Staphylococcus aureus* are the most common cause of mortality and morbidity worldwide. Antimicrobial therapeutic options against infections caused by resistant pathogens, such as methicillin-resistant *S. aureus* (MRSA), are frequently restricted. The clinical problems emphasize the crucial need for novel and effective antibacterial treatments. Nanoparticles (NPs) act as potential antimicrobial agents. They can penetrate the bacterial cell membrane, disrupt important molecular pathways, showing unique antimicrobial mechanisms. Therefore, the antibacterial effect of gold nanoparticles (AuNPs) on *S. aureus* isolates, including MRSA originating from chicken meat, was examined in this study. The antibacterial activity of chemically synthesized AuNPs (20 nm in size) against *S. aureus* isolates was carried out using the broth microdilution method. The minimum inhibitory concentrations (MICs) of AuNPs for the meat-associated isolates were determined at 500 µg/mL and higher. The results of this study present the susceptibility of the *S. aureus* isolates to AuNPs. Further studies are needed investigating the antibacterial activity and efficacy of different gold nanoparticle sizes and concentrations against resistant pathogens.

Keywords: Gold nanoparticles, *Staphylococcus aureus*, MIC, Antibacterial effect

INTRODUCTION

Antimicrobial resistance is becoming increasingly recognized as a major risk to global public health and development threats (WHO, 2023). The overuse and misuse of antimicrobial agents in humans, animals, and plants are primary causes in the development and spread of antimicrobial-resistant pathogenic bacteria (WHO, 2023; Berman et al., 2023). Bacteria that carry resistance genes can propagate either directly among human populations or indirectly by contaminating food, water, or other surfaces with sewage (Woolhouse and Ward, 2013). In particular, direct transmission of antimicrobial-resistant bacteria from food-producing animals to humans can occur from contact with animals and ingestion of animal products, especially when handled or improperly cooked (Woolhouse et al., 2015; Berman et al., 2023).

The emergence of certain bacteria that have developed high resistance to antimicrobials, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant

Enterococcus (VRE), are the most common bacteria in human infections (Gouyau et al., 2021; Moradi et al., 2023). Therefore, developing novel and potent antimicrobials for controlling these bacteria is a major priority (WHO, 2017; Sanchez-Lopez et al., 2020). For instance, recent studies have shown that specific metal nanoconstructs have antibacterial abilities that may be used to treat infectious diseases (Rai et al., 2009; Sanchez-Lopez et al., 2020; Gouyau et al., 2021).

Metal-based nanoparticles are the most widely utilized inorganic nanoparticles and exhibit a promising treatment for resistance to traditional antimicrobials. They do not only demonstrate efficiency against bacteria that have already achieved resistance, but also target a range of biomolecules weakened in the emergence of resistant strains, using completely different mechanisms of action from those described for conventional antimicrobials (Slavin et al., 2017; Sanchez-Lopez et al., 2020).

Gold nanoparticles (AuNPs) among the significant nanomaterials are of particular interest in the area of biomedicine and its uses due to their chemical, electrical, optical, mechanical, and thermal characteristics. These large potentials have raised the possibility of toxicity as a result of interactions with biological tissues and molecules (Pissuwan et al., 2011; Dykman and Khelbstov, 2012; Sani et al., 2021). Several investigations have reported the antibacterial activity of AuNPs against pathogenic bacteria, including *S. aureus* (Li et al., 2011a; Li et al., 2014; Elbehiry et al., 2019; Gouyau et al., 2021). AuNPs have great potential in diagnostic roles, food packaging, drug delivery, photothermal and radiation therapy, biosensing, gene therapy, as well as cancer diagnostics, enzyme fixation, cosmetics, and cell imaging (Pissuwan et al., 2011; Li et al., 2011b; Kong et al., 2017; Elahi et al., 2018; Paidari and Ibrahim, 2021, Sani et al., 2021).

Recently, there has been an increasing amount of research focused on the antibacterial properties of AuNPs, which makes them a good candidate for antibiotic complementation (Sanchez-Lopez et al., 2020; Sathiyaraj et al., 2021; Moradi et al., 2023). Therefore, in this study we aimed to investigate the antibacterial activity of AuNPs against *S. aureus*, which is responsible for food spoilage and foodborne illnesses.

MATERIALS AND METHODS

Bacterial isolates

This study used 13 *S. aureus* recovered from chicken meat samples (breast and leg parts) collected from various supermarkets and butchers. The *S. aureus* isolates from chicken meat were cultured in Tryptic Soy Broth (Merck, Darmstadt, Germany) and incubated overnight at 37 °C. Biochemical tests and PCR analysis for the *S. aureus*-specific genomic fragment (Sa442) and thermonuclease gene (*nucA*) have previously been employed to identify the isolates (Brakstad et al., 1992; Martineau et al., 1998; Götz et al., 2006; Bannerman and Peacock, 2011). Using the *mecA* gene (Milheiriço et al., 2007), these isolates were previously identified as methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) using the *mecA* gene in our previous study (Özdemir, 2022).

Nanoparticles used in the study

The chemically synthesized AuNPs using sodium citrate had a 20 nm particle size and a concentration of 1 mg/mL. The tested concentration of AuNPs ranged from 500 to 0.9766 µg/mL.

Antibacterial activity of AuNPs against *S. aureus* isolates

The antibacterial activity of AuNPs against S. aureus *isolates, including methicillin-resistant* S. aureus *(MRSA), originated from chicken meat, was evaluated by the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), which were achieved by the broth microdilution method (CLSI, 2012; CLSI, 2022). Briefly,* bacterial cells were added to a final concentration of $1x10^6$ CFU/mL. Serial *two-fold dilutions (500-0.9766 µg/mL) were prepared in growth medium Mueller-Hinton broth (Merck, Darmstadt, Germany) in sterile 96-well U-bottom microtiter plates (LP Italiana). The MIC values of* Staphylococcus aureus *ATCC 29213 as a control strain was used in all experiments. After overnight incubation at 37°C, the MIC values of the isolates were determined by measuring the optical density (OD) at 600 nm using a microplate reader (Thermo Electron Corporation, Vantaa, Finland). The MIC is defined as the lowest test concentration of the agent at which bacterial growth is completely inhibited (Andrews, 2001).* The minimum bactericidal concentration (MBC) of *AuNPs* (500, 250 125, 62.5, 31.25 and 15.625 µg/mL) against *S. aureus* isolates were assessed on the Mueller-Hinton agar (Merck, Darmstadt, Germany) in this study. The microtiter plates were incubated at 37°C for 16-20 h. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

The inappropriate and excessive use of antimicrobial agents in humans and animals accelerates the emergence and spread of antibiotic-resistant bacteria, especially multidrug-resistant bacterial pathogens. For instance, methicillin-resistant *S. aureus* (MRSA), one of the most common antibiotic-resistant bacterial pathogens, is resistant to antibiotic treatment and is considered an important threat to human health (Sanchez-Lopez et al., 2020; WHO, 2023). Antibiotic efficacy may be affected by the spread of these strains and their resistance genes. Consequently, there is an crucial need to find and develop novel approaches to fight antimicrobial resistance (WHO, 2017). Pharmaceutical firms involved in the development of non-traditional drugs, particularly those working in the nanotechnology sector, were interested by these new benefits and made investments in the creation of novel nanomaterials that have been shown to be promising agents against bacteria resistant to traditional antibiotics (Sanchez-Lopez et al., 2020).

In recent years, metal-based nanoparticles, including gold, silver, and copper, have shown promising results for combating various resistant microbes (Moradi et al., 2023). In this study, antibacterial effects of gold nanoparticles (AuNPs), 20 nm in size, against *S. aureus* isolates from chicken meat samples were tested by using the broth microdilution method. The values obtained for the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the AuNPs against the isolates are presented in Table 1.

Table 1. MIC values of synthesized AuNPs against *S. aureus* isolated from chicken meat

Isolate	Methicillin Resistance	Au NPs	
		MIC[a]	MBC[b]
C1	MSSA[c]	500	>500
C2	MSSA	>500	>500
C3	MSSA	>500	>500
C4	MSSA	>500	>500
C5	MRSA[d]	500	>500
C6	MSSA	>500	>500
C7	MSSA	>500	>500
C8	MSSA	>500	>500
C9	MSSA	500	>500
C10	MRSA	>500	>500
C11	MSSA	>500	>500
C12	MRSA	500	>500
C13	MRSA	500	>500

[a]MIC, minimum inhibitory concentration (µg/mL); [b] MBC, minimum bactericidal concentration (µg/mL); [c]MSSA, methicillin-sensitive *S. aureus*; [d]MRSA, methicillin-resistant *S. aureus* harboring the *mecA* gene.

The antimicrobial effect of 20 nm AgNPs for the *S. aureus* isolates, including MSSA and MRSA, was found to be 500 µg/ mL and higher. The MIC range of the AuNPs was higher than 500 µg/ mL in 61.5% of the isolates. The image for MBC of AuNPs against the *S. aureus* isolate (C1) is demonstrated in Figure 1.

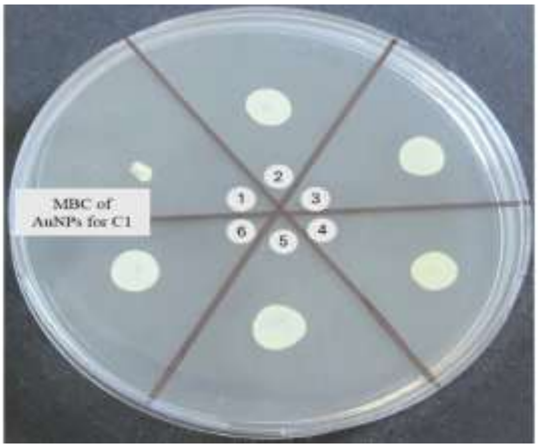


Figure 1. Representative image for the minimum bactericidal concentration (MBC) of AuNPs against *S. aureus* isolate (C1). The numbers 1, 2, 3, 4, 5, and 6 show compound concentrations of 500, 250, 62.5, 31.25, 15.625, and 7.8125 µg/mL, respectively.

In previous studies, the antibacterial activity of AuNPs against *S. aureus* strains was reported (Li et al., 2014; Shamaila et al., 2016; Elbehiry et al., 2019). In the work of Shamila et al. (2016), the antibacterial effects of different sizes of AuNPs were studied against different enteric bacterial human pathogens including *S. aureus* strains. The authors found that the MICs of gold NPs sized 7-34 nm were 3.92 µg/mL, whereas the MICs of gold NPs sized 20-40 nm were 3.98 µg/mL for *S. aureus*. They also reported that larger doses of gold NPs are required for the complete inhibition of *S. aureus* due to their thick peptidoglycan layer. Furthermore, AuNPs effectively inhibited the growth of a variety of multidrug-resistant bacteria, including MRSA (Li et al., 2014). On the contrary, Gouyau et al. (2021) in France revealed that chemically synthesized 12 nm gold nanoparticles had a weak antibacterial activity against the *S. aureus* tested.

CONCLUSION

Antimicrobial alternatives are often limited in treatment to prevent infections caused by antimicrobial-resistant and multidrug-resistant bacteria such as *S. aureus*. Therefore, there is a significant need for novel and effective strategies to combat antibiotic resistance to overcome clinical challenges. Nanoparticles have recently been investigated as potential antimicrobial agents for the treatment of antimicrobial-resistant bacterial pathogens. This study demonstrated the antibacterial activity of gold nanoparticles against the isolates of *S. aureus*, which may pose a potential threat to food safety and human health.

REFERENCES

Andrews JM. 2001. Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy, 48:5-16.

Bannerman TL, Peacock SJ. 2011. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW, eds. Manual of clinical microbiology, Washington, D.C: ASM Press, 308-330.

Berman TS, Barnett-Itzhaki Z, Berman T, Marom E. 2023. Antimicrobial resistance in food-producing animals: towards implementing a one health based national action plan in Israel. Israel Journal of Health Policy Research, 12:18.

Brakstad OG, Aasbakk K, Maeland JA. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. Journal of Clinical Microbiology, 30(7):1654-1660.

Clinical and Laboratory Standards Institute (CLSI). 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Ninth edition, M7-A9, in, Wayne, PA, USA.

Clinical and Laboratory Standards Institute (CLSI). 2022. Performance standards for antimicrobial susceptibility testing; 32nd edition, in, Malvern, PA, USA.

Dykman L, Khelbstov N. 2012. Gold nanoparticles in biomedical applications: recent advances and perspectives. Chemical Society Reviews. 41, 2256-2282.

Elahi N, Kamali M, Baghersad MH. 2018. Recent biomedical applications of gold nanoparticles: A review. Talanta, 184: 537-556

Elbehiry A, Al-Dubaib M, Marzouk E, Moussa I. 2019. Antibacterial effects and resistance induction of silver and gold nanoparticles against *Staphylococcus aureus*-induced mastitis and the potential toxicity in rats. MicrobiologyOpen, 8: e698.

Götz F, Bannerman T, Schleifer KH. 2006. The genera *Staphylococcus* and *Macrococcus*. In: Dworkin M, Falkow S, E, Rosenberg E, Schleifer KH, Stackebrandt E, eds. The Prokaryotes. 3rd ed. New York, N.Y.: Springer, 5-75.

Gouyau J, Duval RE, Boudier A, Lamouroux E. 2021. Investigation of Nanoparticle Metallic Core Antibacterial Activity: Gold and Silver Nanoparticles against *Escherichia coli* and *Staphylococcus aureus*. International Journal of Molecular Sciences, 22, 1905.

Kong F-Y, Zhang J-W, Li R-F, Wang Z-X, Wang W-J, Wang W. 2017. Unique Roles of Gold Nanoparticles in Drug Delivery, Targeting and Imaging Applications. Molecules, 22(9):1445

Li W-R, Xie X-B, Shi Q-S, Duan S-S, Ouyang Y-S, Chen Y-B. 2011a. Antibacterial effect of silver nanoparticles on Staphylococcus aureus. Biometals, 24:135-141.

Li X, Robinson SM, Gupta A, Saha K, Jiang Z, Moyano DF, Sahar A, Riley MA, Rotello VM. 2014. Functional Gold Nanoparticles as Potent Antimicrobial Agents against Multi-Drug-Resistant Bacteria. ACSNano, 8(10): 10682-10686.

Li X, Zhou H, Yang L, Du G, Pai-Panandiker AS, Huang X, Yan B. 2011b. Enhancement of cell recognition in vitro by dual-ligand cancer targeting gold nanoparticles, Biomaterials, 32(10): 2540-2545.

Martineau F, Picard FJ, Roy PH, Quellette M, Bergeron MG. 1998. Species-specific and ubiquitous-DNA-based assays for rapid identification of *Staphylococcus aureus*. Journal of Clinical Microbiology, 36: 618-623.

Milheiriço C, Oliveira DC, De Lencastre H. 2007. Update to the multiplex PCR strategy for assignment of mec element types in *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy, 51, 3374-3377.

Moradi F, Ghaedi A, Fooladfar Z, Bazrgar A. 2023. Recent advance on nanoparticles or nanomaterials with anti-multidrug resistant bacteria and anti-bacterial biofilm properties: A systematic review. Heliyon, 9: e22105.

Özdemir F. 2022. Antimicrobial Resistance, Multilocus Sequence, and spa Typing of *Staphylococcus aureus* Isolated from Retail Raw Meat Products. BioMed Research International, 6035987.

Paidari S, Ibrahim SA. Potential application of gold nanoparticles in food packaging: a mini review. Gold Bull 2021; 54: 31-36

Pissuwan D, Niidome T, Cortie MB. 2011. The forthcoming applications of gold nanoparticles in drug and gene delivery systems. Journal of Controlled Release 149, 65-71.

Rai M, Yadav A, Gade A. 2009. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances, 27: 76-83.

Sanchez-Lopez E, Gomes D, Esteruelas G, Bonilla L, Lopez-Machado AL, Galindo R, Cano A, Espina M, Ettcheto M, Camins A, Silva AM, Durazzo A, Santini A, Garcia ML, Souto EB. 2020. Nanomaterials, 10, 292.

Sani A, Cao C, Cui D. 2021. Toxicity of gold nanoparticles (AuNPs): A review. Biochemistry and Biophysics Reports, 26, 100991.

Sathiyaraj S, Suriyakala G, Gandhi AD, Babujanarthanam R, Almaary KS, Chen T-W, Kaviyarasu K. 2021. Biosynthesis, characterization, and antibacterial activity of gold nanoparticles. Journal of Infection and Public Health, 14, 1842-1847.

Shamaila S, Zafar N, Riaz S, Sharif R, Nazir J, Naseem S. 2016. Gold nanoparticles: An efficient antimicrobial agent against enteric bacterial human pathogen. Nanomaterials (Basel), 6, 1-10.

Slavin YN, Asnis J, Hafeli UO, Bach H. 2017. Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. Journal of Nanobiotechnology, *15*, 1-20.

Woolhouse M, Ward M, Van Bunnik B, Farrar J. 2015. Antimicrobial resistance in humans, livestock and the wider environment. Philosophical Transactions of the Royal Society B: Biological Sciences, 370:20140083. https://doi.org/10.1098/rstb.2014.0083.

Woolhouse MEJ, Ward MJ. 2013. Sources of antimicrobial resistance. Science, 341:1460-1461.

World Health Organization (WHO). 2017. WHO publishes list of bacteria for which new antibiotics are urgently needed. Available online: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

World Health Organization (WHO). 2023. Antimicrobial resistance. Available online: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

EXPLORATION AND CHARACTERIZATION OF INDIGENOUS ALGERIAN SOIL MICROORGANISMS FOR BIOCONTROL AND BIOTECHNOLOGICAL APPLICATIONS

Fatima Idris Khodja [*1], *Nisserine Hamini-Kadar*[1], *Yezli Wassim*[2], *Ismail Saadoun*[3], *Ban Al Jobouri*[3], *Mebrouk Kihal*[1]

1 Laboratory of Applied Microbiology, Department of Biology, Faculty of Natural and Life Sciences, University Oran 1, Algeria

2 Department of Nutrition and Agro-Food Technology, Faculty of Nature and Life Sciences, Ibn Khaldoun University, Tiaret, Algeria

3 Laboratory of Biotechnology, Department of Biology, College of Science, University of Sharjah, United Arab Emirates.

**Correspondence:Email : idriskhodja.fatima@edu.univ-oran1.dz*

ABSTRACT

To discover new biocontrol agents beneficial to agriculture, researchers can explore natural and uncharted ecosystems as potential sources of novel agents for pharmacological and industrial applications. Microorganisms represent the most economically viable and biologically valuable sources for the production of biocontrol compounds. In this study, 42 samples of soil collected from Tifrit forest located in semi-arid region in Saida 34° 50′ 00″ north, 0° 09′ 00″ east (Algeria).The main objective of the study was to assess six *Streptomyces* isolates (T1, T4, T5, T6, T7, and T8) for their enzymatic activity (lipase, protease, amylase, and cellulase) by screening their activities on agar plates and showing a clear zone around the colonies. The isolates were subsequently characterized phenotypically, biochemically, and through PCR analysis and 16S rDNA sequencing. Examination of colony morphology, agar plate culture characteristics, and biochemical reactions suggested that the isolates belong to *Streptomyces* species. The results demonstrated that isolates T1, T4, and T5 exhibited lipase activity by hydrolyzing lipids in egg yolk agar. Also, all isolates displayed protease activity by digesting proteins in skimmed milk agar. Amylase production, indicated by starch digestion in starch casein nitrate agar, was not observed in any of the tested isolates. However, isolates T1, T5, T6, and T8 demonstrated cellulase activity by hydrolyzing cellulose in carboxy methyl cellulose (CMC) agar. Molecular characterization of these isolates was conducted, which included genomic DNA extraction, 16S rRNA gene amplification, and sequencing to confirm the specific *Streptomyces* strains. Genomic DNA was extracted from *Streptomyces* isolates, followed by PCR amplification using Streptomyces-specific primers (RI7F/RI8R and AM45F/AM47R) and universal bacterial primers (27F/1492R) targeting the 16S rRNA gene. All isolates (T1, T4, T5, T6, T7, and T8) produced single PCR bands of approximately 438 bp, 940 bp, and 1480 bp when using RI7F/RI8R, AM45F/AM47R, and 27F/1492R primers, respectively. These results showed the presence of bacterial 16S rRNA and specifically the conserved 16S rRNA region of *Streptomyces* species in all the isolates. The isolates were sequenced using the 27F/1492R primers. BLASTn analysis of the 16S rRNA gene sequences identified isolates T1, T4, T5, and T6 as *Streptomyces aegyptia*, with identity percentages of 99.91%, 99.71%, 99.64%, and 96.7%, respectively. However, isolates T7 and T8 were identified as *Lysinibacillus fusiformis* and *Umezawaea beigongshangensis*, with identity percentages of 99.86% and 99.71%, respectively. The 16S rRNA sequences of these isolates were aligned with similar sequences from the GenBank database, and a phylogenetic tree was constructed. This tree demonstrated that isolates T1, T4, T5, and T6 formed a cluster within the Streptomyces group, while isolates T7 and T8 branched separately, consistent with their identification as belonging to different genera. The findings highlighted the significant potential of these indigenous Algerian forest soil microorganisms, particularly in the discovery of novel enzymes, underscoring their relevance for industrial applications. This research emphasizes the diversity of the microbial population in Algerian soil and the importance of exploring such natural reservoirs for biotechnological advancements.

Key Words: Algerian soil, Enzymatic activity, *Streptomyces*, Tifrit forest.

INTRODUCTION

The actinobacteria phylum stands out as one of the main producers of biologically active substances, used in various fields such as medicine, industry and agriculture **(Genilloud 2017; Trabelsi et al. 2016)**. Actinomycetes, Gram-positive filamentous bacteria, are known to produce a wide range of enzymes for commercial use **(Al-Dhabi et al. 2019)** . In particular, *Streptomyces* species are considered as micro-organisms of great industrial importance due to their ability to generate different enzymes **(Magda et al. 2012, Sarmiento-Vizcaíno et al. 2022)**. Enzymes of microbial origin are particularly prized in the industrial sector because of their remarkable stability, productivity and availability. They are also cost-effective and their production methods are environmentally friendly **(Oumaima and Faouzi 2023)**. From an industrial point of view, their practical and ecological nature makes them even more popular **(Ferreira, Azzoni, and Freitas 2021)**. *Streptomyces spp*. including *S. griseus, S. clavuligerus, S. thermoviolaceus, S. rimouses, and S. thermovulgaris*, which are known for their production of various proteases, exploit both simple and complex molecules as sources of nutrients **(Al-Dhabi, Mohammed Ghilan, and Arasu 2018)**. *Streptomyces* species also secrete a variety of extracellular enzymes. These *Streptomyces* generate extracellular proteases, the production of which is closely linked to sporulation and the formation of aerial mycelium **(Arasu et al. 2019)**. The enzymes most commonly produced by *Streptomyces* species include protease, chitinase, amylase, lipase, xylanase, cellulase and laccase **(Oumaima and Faouzi 2023)**.The extracellular enzymes produced by *Streptomyces* have significant potential to replace the toxic chemicals used in various industries. However, the adoption of these alternatives has not yet been given a wide platform in developing countries. This study was carried out to identify new *Streptomyces* strains capable of producing extracellular enzymes. This research project focuses on exploring the biotechnological potential of six *Streptomyces* strains isolated from Algerian forest soils, highlighting their reservoir of genetic and metabolic diversity for enzyme production. Assessing the enzymatic capacities of these strains is a central objective of this study.

MATERIALS AND METHODS

Isolation of actinomycetes from soil

Soil samples were systematically collected from Tifrit forest, located in Saida city, western Algeria. The collection was performed utilizing the Pochon and Tardieux technique, ensuring consistency in sampling. All samples were extracted from a depth of 15 cm and subsequently dried under aseptic conditions **(Idris Khodja et al. 2023)**. The agar medium dilution method was used for the isolation of actinomycete strains. Samples were diluted in successive series to 10^{-5}, then an aliquot of 0.1 ml was deposited on the surface of starch-casein-nitrate (SCNA) agar medium, enriched with nalidixic acid (20 mg/l) to inhibit the growth of Gram- negative bacteria, and nystatin (50 mg/l) to inhibit the development of fungi **(S.Loqman 2009)** all plates were incubated at 28°C for 5 to 10 days at a rate of three plate per dilution **(Songsumanus et al. 2011)**. Observable colonies formed on the plates were isolated and grown on the same medium, then incubated for a further 10 days. The isolates thus obtained were characterised based on their morphological and cultural properties. Before subculturing, *Streptomyces* isolates were inoculated onto GYM agar medium and stored at 4°C for three months **(Gacem et al. 2020)**. Isolated colonies were subcultured on International Streptomyces Project (ISP: ISP2-ISP7) agar media to obtain pure cultures. In accordance with the protocol of Shirling and Gottlieb (1966), the morphological, biochemical and physiological characteristics of the actinomycete isolates were determined **(Shirling and D. Gottlieb 1966)**. Six isolated colonies of actinomycetes (T1, T4, T5, T6, T7, and T8) were selected for their enzymatic activity (lipase, protease, amylase, and cellulase) by screening their activities on agar plates.

Morphological and physiological analysis of isolates

Macromorphology and cultivation characteristics

Morphological characterisation was carried out by examining the growth behaviour of isolates on different culture media: oat agar (ISP3), inorganic salt-starch agar (ISP4), glycerol-asparagine agar (ISP5), yeast extract-malt extract agar (GYM) and starch-casein agar (SCA). We carried out a visual assessment of characteristics such as the coloration of aerial and substrate mycelia, as well as the production of soluble pigments.

Scanning electron microscope observation

The scanning electron microscope (SEM) examinations were carried out in the laboratories of the Advanced Materials Research Centre at the University of Sharjah, United Arab Emirates.
The spore surfaces of active actinomycetes isolates were examined using a scanning electron microscope (SEM) at magnifications ranging from 5000X to 25000X. To prepare the samples, buffer was placed on 21-day cultures of each isolate grown on oat agar (ISP3). The buffer was then subjected to a sputter coating process using a Quorum Technology Mini Sputter Coater, with a target composition of gold (80%) and palladium (20%), for a duration of 2 minutes, resulting in the deposition of approximately 100 A° of coating material. The gold sputterer was operated at 1 kV, with a plasma current of 18 mA and a chamber pressure of 10-2 mbar. After the coating process, the samples were observed using a Tescan VEGA XM variable-pressure

SEM, with a maximum accelerating voltage of 30 kV. Images were viewed on a computer screen and an Oxford Instruments X-Max 50 EDS detector (system without LN2) with 125 eV resolution was used for further analysis. The surface structures of the spores were classified as smooth (sm), spiny (sp), warty (wa) or hairy (ha). We took a 5-micron and a 3-micron photo of each sample.

Preparation of *Streptomyces* spore suspension

The selected *Streptomyces* isolates were suspended in sterile polystyrene tubes containing 3 ml of distilled water, in order to obtain a spore suspension with a concentration of 10^7 spores/ml **(Bonaldi 2014; Shepherd et al. 2010)**.

Detection of amylase production

A 25µl volume of the spore suspension of pure actinomyces isolates was grown on starch-casein-nitrate agar (SCNA) and incubated at 28°C for 4 days. The plates were then flooded with an iodine solution and left for 30 minutes, before being washed with distilled water **(dos Santos et al. 2012)**. Bacterial colonies producing amylase show a light zone against the black colour of the coloured starch.

Detection of proteolytic activity

A 25µl volume of the spore suspension of pure Actinomyces isolates was inoculated onto agar plates containing skimmed milk casein (Himedia, India) and incubated at 28°C for 4 days. The appearance of thinning zones around the colonies revealed proteolytic activity of the isolates, indicating hydrolysis of the casein **(Sharmin et al. 2005)**.

Detection of lipolytic activity

A 25µl volume of the spore suspension from the pure isolates was streaked onto egg yolk agar plates containing Baird-Parker agar base (Oxoid, UK) supplemented with 50% egg yolk emulsion (Mast Diagnostics, UK). The dishes were then incubated at 28ºC for 4 days **(Magda et al. 2012)**. The appearance of light zones around the colonies indicates lipolytic activity of the isolates, resulting from hydrolysis of the lipids present in the egg yolk.

Detection of cellulolytic activity

A 25µL volume of a spore suspension of pure isolates was inoculated onto agar plates containing carboxymethyl cellulose (CMC), followed by incubation at 28°C for 4 days. After incubation, the plates were flooded with 0.1% Congo red solution and left in contact for 15-20 minutes, then rinsed with 1 ml NaCl solution and incubated at 4°C overnight. The formation of clear zones around the colonies, in contrast to the red colour of the non-hydrolysed medium, revealed the cellulolytic activity of the isolates, indicating the hydrolysis of cellulose **(Saratale et al. 2014)**.

Genomic Sequencing

Genomic DNA from isolates was extracted using the Promega Wizard® Genomic DNA Purification Kit from Promega, USA, following the manufacturer's instructions designed for Gram-positive organisms. The quality and quantity of the DNA obtained were assessed by 1% agarose gel electrophoresis and NanoDrop (Thermo Fisher Scientific). A 1 ml aliquot of a 7-day-old culture was transferred to an Eppendorf tube and centrifuged at 13,000 rpm for 2 minutes. After removal of the supernatant, the cells were resuspended in 480 µl of 50 mM EDTA. After homogenisation, the mixture was incubated at 37°C for 30-60 minutes, followed by centrifugation at 13,000 rpm for 2 minutes. Next, 600 µl of nuclear lysis solution is added, and the cells are carefully resuspended by pipetting. The DNA is then purified according to the manufacturer's instructions, using the Wizard® Genomic DNA Purification Kit.

PCR primers specific to actinomycetes

Two sets of primers were used in this study for the molecular identification of actinomycetes producing the most active metabolites. The first series (AMF and AMR) had the following sequences:
AM45F_5‘-GTG AGT CCC CAG ATC ACC CCG AAG-3’ and AM47R_5‘-GTG GGC AAT CTG CCC TTG CAC TCT-3’ respectively, and amplified a Streptomyces-specific target sequence of 438 bp. The second sets R17/R18 (R1) had the following sequences:
R17_5‘-GTGAAAGCCCGGGGCTTAAC-3’ and R18_5'-CACCACCACAAGGGGCA, respectively, they amplified a Streptomyces-specific target sequence of 438 bp. The third sets (Actino-F (AO1) and Actino-R (AO2)) had the following sequences:
2- FC27_5‘-AGA GTT TGA TCC TGG CTC AG-3’ and RC1492 _5‘-TAC GGC TAC CTT GTT ACG ACTT-3’, respectively, and amplified a specific region within the 16S rRNA of actinomycetes with a size of 1,490 bp. The primers were synthesised by Invitrogen Life Technologies (USA).

Amplification of 16S rRNA gene fragment

The reaction mixture for PCR amplification was prepared in a volume of 25 µl containing the following: Actino-F; 10 µM, Primer Actino-R; 10 µM, Hot Start 2X Taq master mix (BioLabs, New England), template DNA and Nuclease-Free water (BioLabs, New England) was used to bring the reaction volume to 25 µl. A similar mix was used for the other two primer sets.
PCR amplification was performed in thin-walled, nuclease-free 0. 2 ml thin-walled nuclease-free PCR tubes (Treff Lab, Switzerland) using the iCycler thermal cycler (Bio-Rad, USA) programmed as follows: Initial denaturation at 95 ˚C for 30 seconds, followed by a second denaturation at 95 ˚C for 30 seconds, annealing at 55 ˚C (for (Actino-F (AO1) and Actino-R (AO2)) for 40 seconds, extension at 68˚C for 1:30 min for 30 cycles and further extension at 68˚C for 5 minutes.

Finally, tubes were stored at 4 ˚C for direct use, or stored at -20 ˚C until use. The program was run for all primers in a similar manner, but only changing the annealing temperature for each set of primers, with the annealing temperature for the R17/R18 set (R1) being 64 ˚C and for the set (AMF and AMR) being 62 ˚C.

Electrophoresis and photography

The presence of DNA in the PCR products was verified by a standard electrophoresis procedure using 2% (w/v) agarose gels (PROMEGA, USA) and detected by ethidium bromide (EB) staining (Acros Organic, USA) at a final concentration of 0.5µg/ml. Electrophoresis was performed at 100 volts for 40 minutes. The size of PCR products was estimated using a 1 Kb DNA ladder (Promega, USA). Gels were visualised and photographed using Gel Doc-It-310 (Imaging System, UVP-USA).

Sequencing

ExoSAP-IT treatment

The ExoSAP-IT treatment begins by adding 1 µl (variable depending on the product) of PCR product, 2 µl of ExoSAP-IT reagent and 4 µl of NFW (nuclear free water) to a labelled PCR tube. The PCR tubes are then centrifuged using a mini-centrifuge for a few seconds to collect any drops on the walls. The tube was then incubated at 37°C for 15 minutes, followed by 15 minutes at 80°C by placing it in the PCR machine and running it through the ExoSAP-IT programme.

Sequencing reaction cycle

After the incubation period, two PCR tubes are labelled for each sample; one with the unknown sample number followed by the letter F and the other with the unknown sample number followed by the letter R. To both tubes, 7 µl of the ExoSAP-IT-treated sample from the previous step, 4 µl of forward primer (1uM/ul), 4 µl of reverse primer (1uM/ul), 2 µl of BigDye sequencing buffer and 1 µl of ready-to-use premix are added. Some NFW (nuclease-free water) is added to reach a total volume of 20 µl.
The PCR tubes are centrifuged using a mini-centrifuge to collect the drops on the sides, then placed in the PCR machine and run using the Cycle Sequencing PCR program with the BigDye terminator v3.1 sequencing kit. Finally, the samples are stored at -20°C until the next step.

Sequence clean-up

To completely remove excess terminations, ethanol/EDTA precipitation was performed. Sequencing reactions were precipitated in 96-well reaction plates by first removing the plate from the thermal cycler and wringing it out briefly and then adding 2 µL of 125 mM EDTA and 2 µL of 3 M sodium acetate to each well, ensuring that the solutions reached the bottom of the wells. Next, 50 µL of 100% ethanol was added to each well, and the plate was sealed with foil tape and mixed by inverting four times before incubating at room temperature for 15 minutes.

The plate was centrifuged at 4°C and 1650 rpm for 45 minutes using a Beckman Allegra 6A centrifuge equipped with a GH-3.8A rotor. After centrifugation, 70 µL of 70% ethanol was added to each well, followed by rotation at 4°C and 1650 xg for 15 minutes. Finally, the samples were resuspended in injection buffer, covered with aluminium foil and stored at 4°C. The next step should be carried out immediately after centrifugation, or the plate should be centrifuged for a further 2 minutes before proceeding. Once the cleaning process was complete, the samples were placed in a 96-well optical reaction plate and cycled for two hours on the sequencer to obtain the corresponding chromatograms. Bioinformatics analysis and identification were then carried out.

RESULTS AND DISCUSSION

Morphological, cultural and physiological characterisation of selected isolates

According to the results obtained, the ISP, GYM and CSA culture media proved effective for isolating actinobacteria from forest soils. The following figures show the cultural and morphological characteristics of the selected isolates:

Figure 1: Morphological characteristics of the isolates T1, T4, T5, T6, T7, T8 (on ISP3 plates) from left to right

Figure 2: Morphological characteristics of the isolates T1, T4, T5, T6, T7, T8 (on CSA plates) from left to right

Figure 3: Morphological characteristics of the isolates T1, T4, T5, T6, T7, T8 (on GYM plates) from left to right

Microscopic observation results

Microscopic examination revealed that all isolates had a branched filamentous structure, which may or may not be segmented, and sometimes fragmented. This morphology clearly associates them with actinomycetes.

After being grown on ISP3 medium at 28°C for 21 days, micromorphological examination of strains T1, T4, T5, T6, T7, T008, using a scanning electron microscope, revealed spaces between the spores and made it possible to visualise the distinctive sporophores of *Streptomyces*.

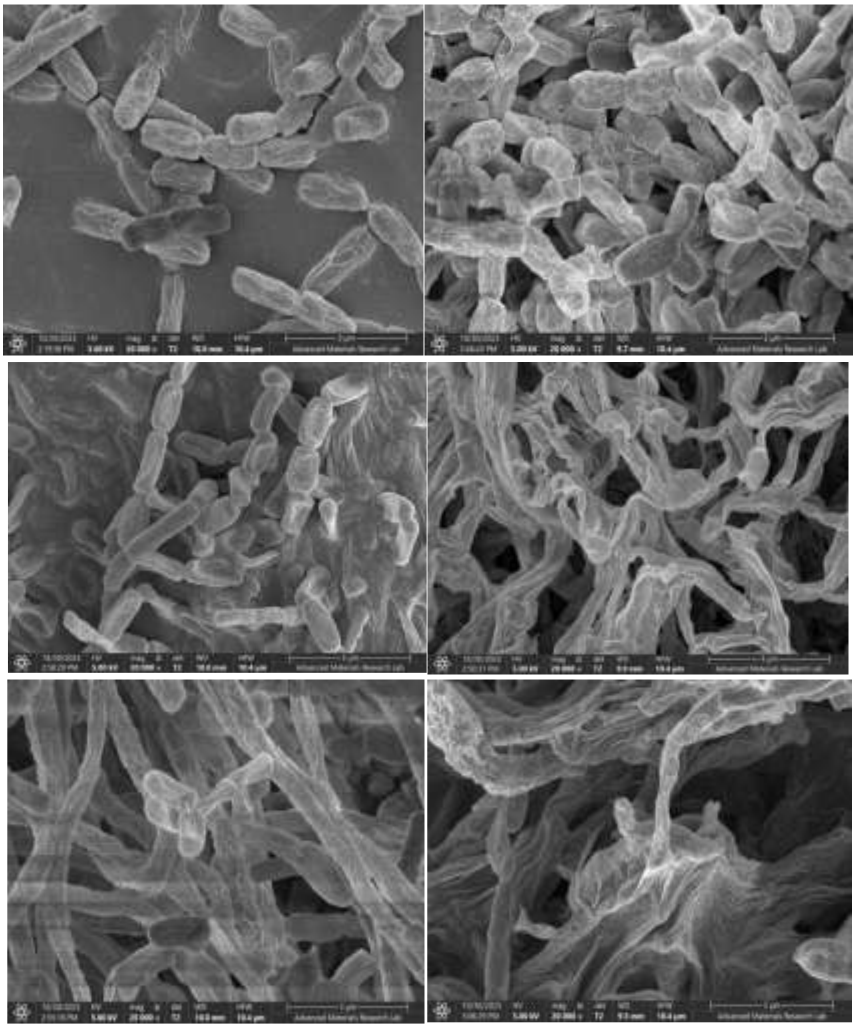


Figure 4: Spore surface morphology of isolates T1, T4, T5, T6, T7, T8 visualized by SEM at 10,000 from left to right

Lipase-producing actinomycetes

Microbial lipases are attracting growing interest as a result of rapid advances in enzyme technology **(Magda et al. 2012)**. They are now the third most widely marketed enzyme in terms of sales volume, after proteases and amylases **(Hasan, Shah, and Hameed 2006)**. In our study, the formation of clear zones around the colonies testifies to the lipolytic activity of the isolates, revealed by the hydrolysis of lipids. Of the six isolates evaluated, isolates T001, T004 and T005 showed significant lipase activity, confirmed by the digestion of lipids present in egg yolk agar, resulting in the appearance of clear zones around their colonies. In contrast, isolate T008 showed no lipase activity.

Figure 5: Assessment of lipolytic activity (clear zone around strains T1, T4 and T5, no zone around T8)

Protease-producing Actinomyces

Actinomycetes are major producers of proteases, enzymes essential to many biological processes. Among the various proteases they secrete, some play a key role in critical extracellular processes linked to their development. These processes, regulated by specific inhibitors, lead to the degradation of the biomass of the substrate mycelium, thereby promoting aerial growth and sporulation **(Chater 2016)**. The presence of clear zones around the colonies indicates proteolytic activity of the isolates, due to the hydrolysis of casein. Of the isolates tested, T1, T4, T5, T6, T7 and T8 showed protease activity by degrading the protein present in skimmed milk agar, which generated clear zones around their colonies.

Figure 6: Evaluation of proteolytic activity (clear zone around strains: T1, T4, T5, T6, T7 and T8)

Amylase-producing Actinomyces

Amylase-producing bacterial colonies were characterised by the formation of clear zones on starch agar. However, none of the isolates tested showed signs of amylase production, as evidenced by the absence of clear zones around colonies on starch, casein and nitrate agars.

Cellulase-producing Actinomyces

Bacterial colonies producing cellulase can be distinguished by the formation of clear zones on carboxy-methylcellulose (CMC)-stained agar. Of the isolates tested, T001, T005, T006 and T007 showed cellulolytic activity by degrading CMC, as evidenced by the presence of clear zones around their colonies on the CMC plates.

Figure 7: Evaluation of cellulosic activity (clear zone around strains: T1, T5, T6 and T7)

Molecular characterisation of isolates

Isolates were subjected to molecular characterisation involving genomic DNA extraction followed by Nanodrop spectrophotometric analysis. The results obtained for each isolate, including the optical density ratio (A260/280) and DNA concentration (ng/µl), are presented in the table below.

Table 1: Nanodrop results for each isolate

Isolates	Density A260/280	Concentarion ng/µl
T1	1.83	14.5
T4	1.52	11.6
T5	1.81	67.7
T6	1.87	141.5
T7	0.97	1.6
T8	1.71	14.9

Phyolgenetic Tree

MEGA version 7.0 was applied to generate a phylogenetic tree using the Neighbor-Joining method **(Saitou and Nei 1987)**. Bootstrap sampling, with 1000 replications, was used to estimate branch confidence values **(FELSENSTEIN 1985)**.

Figure 8 presents a phylogenetic analysis based on 16S rRNA sequences, highlighting the evolutionary relationships between the isolates studied. This analysis revealed that isolate T1 has a close genetic affinity with *Streptomyces aegyptia*, suggesting a common evolutionary lineage and significant genetic similarity. Similarly, isolate T4 showed a genetic relationship with a *Streptomyces sp*. strain, indicating its taxonomic membership of the wider *Streptomyces* genus. In addition, isolate T5 showed significant genetic similarities with *Umezawaea beigonoshangensis*, underlining its potential taxonomic association with this species. In addition, isolate T6 shares genetic characteristics with another *Streptomyces sp*. strain, further strengthening its taxonomic classification. Genetic analysis revealed that isolate T7 shares genetic similarities with *Lysinibacillus sp*, further clarifying its taxonomic categorisation within this bacterial genus. Finally, isolate T8 showed genetic affinity with *Umezawaea endophytica,* suggesting a significant evolutionary link between these two distinct isolates.

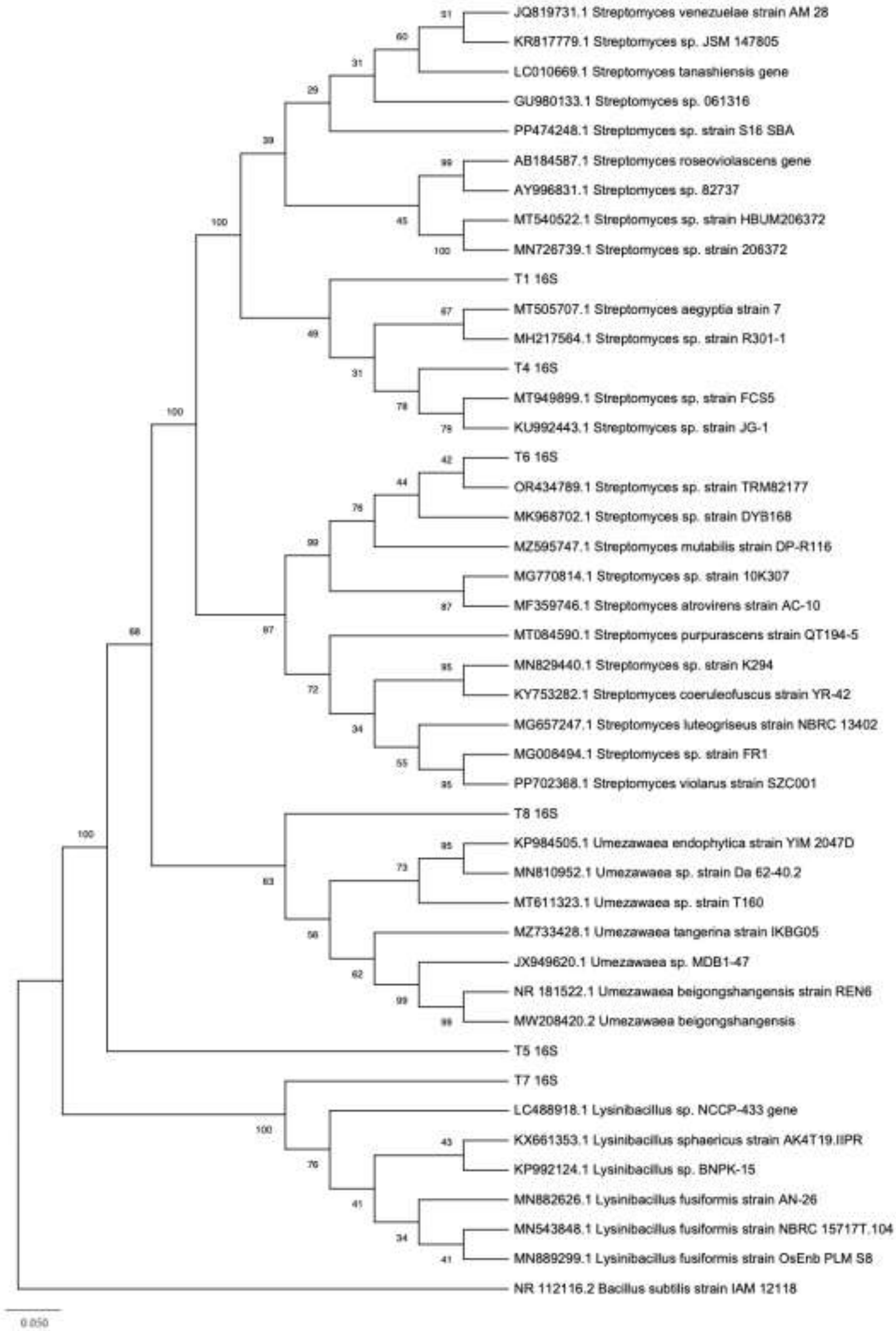


Figure 8: Phylogenetic tree constructed by the neighbour-joining method using blast results

CONCLUSION

Following in-depth analysis and characterisation of the *Streptomyces* isolates in this study, a number of significant results were obtained. The isolates showed a remarkable variety of colony morphologies and aerial mycelium colours, reflecting their intrinsic diversity. In addition, some samples showed significant production of soluble pigments. Detailed examination of the sporophores revealed distinct structures among the isolates, highlighting their heterogeneity.
In addition, assessment of hydrolytic enzyme production revealed significant variation in enzyme activities among isolates, with lipase, protease and cellulase activities detected in varying combinations. It should be noted that none of the isolates showed the ability to synthesise amylase. DNA analysis and the construction of a phylogenetic tree for molecular characterisation revealed genetic relationships and potential evolutionary lineages between isolates. This phylogenetic analysis revealed affinities with established species within the *Streptomyces* genus as well as with other bacterial groups, shedding light on their taxonomic classification.
This study highlights a promising enzymatic activity in actinobacteria isolated from unexplored forest soil in a semi-arid region of Saïda in western Algeria. Further exploration of the enzymatic activities, genetic characteristics and identification of metabolites that can be synthesised by these isolates could provide valuable information for applications in biotechnology, agriculture and pharmaceuticals.

ACKNOWLEDGEMENTS

We would like to thank the team of researchers and technicians in the biotechnology laboratories of the Department of Applied Biology at the University of Sharjah (United Arab Emirates). We would also like to thank Professor Nouar Tabet, Dean of the Faculty of Science, and Dr Hussein Alawadi, Director of the Advanced Materials Research Centre at the University of Sharjah, for their invaluable assistance.
The authors declare that there is no conflict of interest.

BIBLIOGRAPHY

Al-Dhabi, Naif Abdullah, Abdul Kareem Mohammed Ghilan, Galal Ali Esmail, Mariadhas Valan Arasu, Veeramuthu Duraipandiyan, and Karuppiah Ponmurugan. 2019. "Environmental Friendly Synthesis of Silver Nanomaterials from the Promising *Streptomyces Parvus* Strain *Al-Dhabi-91* Recovered from the Saudi Arabian Marine Regions for Antimicrobial and Antioxidant Properties." *Journal of Photochemistry and Photobiology B: Biology* 197:111529. doi: 10.1016/j.jphotobiol.2019.111529.

Al-Dhabi, Naif Abdullah, Abdul Kareem Mohammed Ghilan, and Mariadhas Valan Arasu. 2018. "Characterization of Silver Nanomaterials Derived from Marine *Streptomyces Sp. Al-Dhabi-87* and Its in Vitro Application against Multidrug Resistant and Extended-Spectrum Beta-Lactamase Clinical Pathogens." *Nanomaterials* 8(5):1–13. doi: 10.3390/nano8050279.

Arasu, Mariadhas Valan, Selvaraj Arokiyaraj, Ponnuswamy Viayaraghavan, Thangasamy Sujin Jeba Kumar, V. Duraipandiyan, Naif Abdullah Al-Dhabi, and K. Kaviyarasu. 2019. "One Step Green Synthesis of Larvicidal, and Azo Dye Degrading Antibacterial Nanoparticles by Response Surface Methodology." *Journal of Photochemistry and Photobiology B: Biology* 190:154–62. doi: 10.1016/j.jphotobiol.2018.11.020.

Bonaldi, Maria. 2014. "Streptomycetes as Biological Control Agents and Plant Growth-Promoting Bacteria.". Thèse de doctorat, Université de Milan,115p.

Chater, Keith F. 2016. "Recent Advances in Understanding Streptomyces." *F1000Research* 5(0):1–16. doi: 10.12688/f1000research.9534.1.

FELSENSTEIN, JOSEPH. 1985. "Confidence Limits on Phylogenies: An Approach Using the Bootstrap." *Evolution* 39(4):783–91.

Ferreira, Rafael G., Adriano R. Azzoni, and Sindelia Freitas. 2021. "On the Production Cost of Lignocellulose-Degrading Enzymes." *Biofuels, Bioproducts and Biorefining* 15(1):85–99. doi: 10.1002/bbb.2142.

Gacem, Mohamed Amine, Ould-El-Hadj-Khelil, Aminata, Badreddine Boudjemaa, and Joachim Wink. 2020. "Antimicrobial and Antioxidant Effects of a Forest Actinobacterium V002 as New Producer of Spectinabilin, Undecylprodigiosin and Metacycloprodigiosin." *Current Microbiology* 77(10):2575–83. doi: 10.1007/s00284-020-02007-1.

Genilloud, Olga. 2017. "Actinomycetes: Still a Source of Novel Antibiotics." *Natural Product Reports* 34(10):1203–32. doi: 10.1039/c7np00026j.

Hasan, Fariha, Aamer Ali Shah, and Abdul Hameed. 2006. "Industrial Applications of Microbial Lipases." *Enzyme and Microbial Technology* 39(2):235–51. doi: 10.1016/j.enzmictec.2005.10.016.

Idris Khodja, Fatima, Nisserine Hamini-kadar, Mohamed Amine Gacem, and Mebrouk Kihal. 2023. "Propriétés Antifongiques et Solubilisantes de Phosphate in vitro des métabolites secondaires produits par une *Streptomyces sp T002* rare." *Agrobiologia* 13:3384–92.

Magda, M. Aly, Tork Sanaa, M. Al Garni Saleh, and Nawar Lubna. 2012. "Production of Lipase from Genetically Improved *Streptomyces Exfoliates LP10* Isolated from Oil-Contaminated Soil." *African Journal of Microbiology Research* 6(6):1125–37. doi: 10.5897/ajmr11.1123.

Oumaima, Beroigui, and Errachidi Faouzi. 2023. "*Streptomyces* at the Heart of Several Sectors to Support Practical and Sustainable Applications: A Review." *Progress in Microbes and Molecular Biology* 6(1):1–46. doi: 10.36877/pmmb.0000345.

Souad Loqman. 2009. "La lutte biologique contre la pourriture grise de la vigne: Isolement, caractérisation de souches de bactéries Actinomycétales antagonistes à partir des sols rhizosphériques de vignes saines sauvages d'origine marocaine". Thèse de Doctorat. Universite de Reims Champagne-Ardenne. 253p.

Saitou Naruya, and Masatoshi Nei. 1987. "The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees" Mol. Biol. Evol 4(4):406–25.

Dos Santos, Édilla Ribeiro, Zozilene Nascimento Santos Teles, Núria Mariana Campos, Diogo Angeli Jacinto de Souza, Aline Simões da Rocha Bispo, and Rodrigo Pires do Nascimento. 2012. "Production of α-Amylase from *Streptomyces Sp. SLBA-08* Strain Using Agro-Industrial by-Products." *Brazilian Archives of Biology and Technology* 55(5):793–800. doi: 10.1590/S1516-89132012000500020.

Saratale, Ganesh D., Rijuta G. Saratale, and Sang Eun Oh. 2014. “Production and Characterization of Multiple Cellulolytic Enzymes by Isolated *Streptomyces Sp*. MDS.” *Biomass and Bioenergy* 47:302–15. doi: 10.1016/j.biombioe.2012.09.030.

Sharmin. S,Towhid Hossain and M.N., Anwar. 2005. “Isolation and Characterization of a Protease Producing Bacteria *Bacillus Amovivorus* and Optimization of Some Factors of Culture Conditions for Protease Production.” *Journal of Biological Sciences* 5(3):358–62. doi: 10.3923/jbs.2005.358.362.

Shepherd, Micah D., Madan K. Kharel, Mary A. Bosserman, and Jrgen Rohr. 2010. “Laboratory Maintenance of *Streptomyces* Species.” *Current Protocols in Microbiology* (SUPP.18):1–8. doi: 10.1002/9780471729259.mc10e01s18.

Shirling and D. Gottlieb. 1966. “Methods for characterization of streptomyces species.” *International Journal o f Systematic Bacteriology* 16 (1946):340.

Songsumanus, Apakorn, Somboon Tanasupawat, Chitti Thawai, Khanit Suwanborirux, and Takuji Kudo. 2011. “*Micromonospora Humi Sp. Nov*., Isolated from Peat Swamp Forest Soil.” *International Journal of Systematic and Evolutionary Microbiology* 61(5):1176–81. doi: 10.1099/ijs.0.024281-0.

Trabelsi, Ines, Daniel Oves, Angel Manteca, Olga Genilloud, Abdullah Altalhi, and Mohamed Nour. 2016. “Antimicrobial Activities of Some Actinomycetes Isolated from Different Rhizospheric Soils in Tunisia.” *Current Microbiology*. doi: 10.1007/s00284-016-1053-5.

COMFREY (*Symphytum officinale* L) ETHANOLIC EXTRACT AS A POTENTIAL COSMETIC INGREDIENT CHEMICAL AND PHYSICAL EVALUATION

Marija BANOŽIĆ, Maja ROTIM, Helena BREKALO, Danijela PETROVIĆ*

Faculty of Agriculture and Food Technology, University of Mostar, Biskupa Čule bb, 88000 Mostar, Bosnia and Herzegovina
*Corresponding author

Comfrey (*Symphytum officinale* L.) has a centuries-old tradition as a medicinal plant. Today, multiple randomized controlled trials have demonstrated the efficacy and safety of comfrey external preparations. In recent years, significant research has focused on evaluating the chemo-biological profile of comfrey. The aim is to broaden the medicinal applications of this genus to encompass new pharmacological uses and to discuss the toxicological effect of comfrey oral and dermal preparations considering pyrrolizidine content. Nevertheless, to date there are no previous report on volatile organic compound profile using gas chromatography coupled with mass spectrometry. According to the European Union herbal monograph, *Symphytum officinale* L., radix herbal preparation should be prepared using ethanol 65% (*V/V*) as extraction solvent. Prepared in this way, the liquid extract was tested for Volatile organic compounds profile, dynamic viscosity, pH value and antiradical activity. Using gas chromatography coupled with mass spectrometry, a total of 22 compounds were identified: Ethyl 3-(N-butylacetamido) propanoate, C-methyl-scyllo-inositol and lanosterol most abundant one. Conversely, 65% ethanolic extract allantoin content was under the limit of detection. The kinematic viscosity of the obtained extract was 2.25 ±0.06 mm^2/s while pH value was 6.55. Antiradical activity, measured with the DPPH test was 59.96 ±0.45%. In conclusion, the 65% ethanolic extract prepared according to European Union herbal monograph standards showed low levels of allantoin, indicating a deviation from traditional expectations while at the same time, the extract demonstrated a wide range of volatile organic compounds and high antiradical activity, suggesting potential therapeutic benefits beyond its traditional uses.

Keywords: comfrey; allantoin; volatile organic compounds

INTRODUCTION

Comfrey (*Symphytum officinale* L. is a perennial herbaceous plant *Boraginaceae* family. It has characteristic pale purple flowers and long rough leaves (Melnyk et al., 2024). Comfrey has developed a spindle-shaped branched root, which is considered the most valuable part of the plant. Allantoin is the most significant pharmacologically active constituent in comfrey root, which accounts for 0.6-0.8% of the dry weight (Araujo et al., 2012). Besides allantoin, roots contain mucous polysaccharides (29%), fructose and glucose, phenolic acids like rosemary acid (up to 0.2%), chlorogenic acid (0.012%), caffeic acid (0.004%), α-hydroxide of caffeic

acid, glycopeptides, amino acids, and vitamins and minerals. Allantoin and Rosmarinic acid are usually considered as key compounds responsible for its bioactivities but other responsible compounds and molecular mechanisms have yet to be fully investigated (Staiger, 2012; Savić et al., 2015).
Comfrey root has been part of traditional medicine due to its analgesic and anti-inflammatory activity for centuries (Kucera et al., 2018). Today, multiple randomized controlled trials have demonstrated the efficacy and safety of comfrey external preparations with the purpose of repairing damaged tissue. This mechanism involves three stages: inflammation, proliferation, and remodeling, which occur gradually and dynamically (Araujo et al., 2012). Formulations prepared from the comfrey root are well-established and marketed in more than 10 countries. Mostly, those formulations are marketed for osteoarthritis and blunt injuries in the form of gels and emulsions (Staiger, 2012). Moreover, comfrey root formulations have been evaluated as safe and effective alternatives to topical non-steroidal anti-inflammatory drugs (Smith et Jacobson, 2011). On the other hand, comfrey root is well known for its toxic pyrrolizidine content (0.013-1.2%). Many studies have reported hepatoxicity, carcinogenicity and mutagenicity in the acute/chronic ingestion of pyrrolizidine-containing plants. Regarding the safety of comfrey preparations, even when they are intended primarily for topical application, the primary focus lies in the presence of pyrrolizidine alkaloids, which have the ability to be absorbed through the skin and subsequently activated metabolically (Frost et al., 2014). Therefore, many studies focus on the evaluation of the content of pyrrolizidine alkaloids while data on the content of other compounds are very limited. Trifan et al. (2018) stated that comfrey root preparations distributed on the European market are usually delivered from cultivars that do not contain pyrrolizidine alkaloids, or those formulations are prepared with root extract from pyrrolizidine alkaloids-depleted material. Therefore, there is a need for chemical evaluation of other compounds present in comfrey root in order to broaden the medicinal applications of this genus to encompass new pharmacological uses and to discuss the toxicological effect. The physical and chemical properties of comfrey extract can help to understand bioactive compounds' release properties and skin permeability properties. Furthermore, the physic-chemical properties of comfrey root extract can serve as a determination factor for internal and external purposes of comfrey root.

MATERIALS AND METHODS

Extraction procedure

Comfrey extracts are prepared in accordance with the "European Union herbal monograph" using maceration during 2 weeks, the ratio of solid to liquid was 1/10 g/ml with ethanol in water solution (0-60% V/V). After extraction, extracts were centrifuged (5000 rpm, 5min) filtered through filter paper and stored until analysis.

Viscosity and pH value of extract

The viscosity of extracts was measured using a Micro-Ubbelohde viscometer (DIN 55 350) immersed in a 21 °C water bath. Every sample was measured in three parallels and the results are expressed as the mean value.
The pH value was determined using a Mettler Toledo pH meter. Every sample was measured in three parallels and the results are expressed as the mean value.

Determination of antiradical activity

The previously described spectrophotometric method (Jakobek et al., 2007) with DPPH (2,2-diphenyl-1-picrylhydrazyl) was used to evaluate antioxidant activity. Namely, the extracts were filtered through a nylon filter pore size of 0.4μm and diluted to a concentration of 1 mg/ml using methanol. Measurement solutions were prepared with the addition of 1.2 ml of extract and 0.5 ml of 0.2 mM freshly prepared DPPH solution. All the measurements were done in triplicate at the wavelength of 517 nm.

Allantoin determination

The content of allantoin was determined using the colorimetric method described by Borchhers (1977). Al the measurements were done in triplicate at the wavelength of 520 nm.
Allantoin presence was confirmed using LC-MS/MS with multiple reaction monitoring (MRM) Agilent Ultivo triple quadrupole mass spectrometer system, with following conditions: Eluent: water: acetonitrile start: (90%:10%) than increasing the acetonitrile concentration from 10% to 20% in 2 minutes, then increasing the acetonitrile to 90% in 2.5 minutes., holding 90% acetonitrile for 0.3 minutes. The measurement time was 5 minutes. The temperature of analysis was set at 25 °C. The injection volume was 1uL.

GC-MS Analysis

10 of extract μL was transferred to a closed chromatographic vial and evaporated on a heating plate. Using a gas-tight syringe gaseous sample was analyzed using a Bruker 436-GC gas chromatograph coupled with Bruker SCION SQ (single quadruple, electron ionization) mass spectrometer (Durham, UK). The estimations were duplicated. The column (BR-5 ms; 0.25 nm × 30 m, df = 0.25 μm) operated at the following temperature regime: 50 °C (2 min) at the temperature rate increase of 10 °C/min up to 170 °C (0 min), then at 25 °C to 280 °C (5 min). Sample separation was set for 1:20, helium was used as the carrier gas. The flow of the mobile phase was 1.0 mL/min, and ionization energy was 70 keV. Scanning was done in the 50-500 m/z range. Chromatographic signals have been identified by comparing them to mass spectra from the National Institute of Standards and Technology's 11 collection. The area under particular chromatographic peaks was determined using a computer program installed in the chromatograph.

RESULTS AND DISCUSSION

The physic-chemical properties of comfrey extracts prepared with different ethanolic solutions are shown in Table 1. All obtained extracts had a characteristic aroma and dark brown color. pH value was in the range 4.65-6.55. The lowest pH value had extract prepared with 5% ethanol while the highest pH value was obtained with 65% ethanol. Similar values were obtained in a study (Araujo et al., 2012) where a pH value of 6.20 for commercial extract and of 6.90 for laboratory-prepared samples were determined. On the other hand, Chen et al. (2018) obtained something higher pH values using (7.15) using green extraction techniques. The obtained data in Table 1 suggest that all extracts were in accordance with the European pharmacopeia specification. Moreover, they were under the ideal range (4.5-7) for cosmetic preparations stability. Dynamic viscosity was in a range from 2.23 to 10 mm^2/s following a linear increase of water content in a solvent. These phenomena are probably related to higher allantoin and polysaccharide solubility in water than in ethanol. Generally, cosmetic formulations with lower viscosity are considered as better lubricant and better moisturizers.

Table 1: Dynamic viscosity and pH value of comfrey root extracts

Extract	%EtOH	pH value	Dynamic viscosity (mm^2/s)
1.	65.00	6.55 ±0.06	2.25 ±0.06
2.	45.00	6.49 ± 0.04	6.11 ±0.15
3.	25.00	6.45 ±0.11	6.77 ±0.41
4.	5.00	4.65 ±0.01	8.55 ±0.12
5.	0.00	6.28 ±0.01	10.00 ±0.1

According to Table 2, the antioxidant activity of obtained extracts was in the range from 30.39% (water extract) to 92.89% (45% *v/v* EtOH). Other studies also evaluated the antioxidant activity of comfrey root extract. Savić et al. (2015) found that the antioxidant activity of comfrey root extract (concentration of 0.15 mg/cm^3) is above 80% and it is slowly increased with increasing concentration. Simultaneously, the antioxidant activity of the allantoin standard compound was evaluated. It was shown that Allantoin in concentrations up to 0.15 mg/cm^3 doesn't possess antioxidant activity. On that account, allantoin is not responsible for antioxidant activity. Probably antioxidant activity is caused by the phenolic compound present in the extract. Evaluating results from Table 2 it was shown that water extract had the lowest antioxidant activity due to water polar properties which favor extraction of allantoin and pectin. Božić et al. (2019) compared different solvents (water, methanol, dichloromethane) in the extraction of antioxidants from comfrey root. Water extract (concentration 33 µg/mL) had an antioxidant activity of 8.33 methanolic extract (concentration of 167 µg/mL) had an antioxidant activity of 10.23% while dichloromethane extract did not possess antioxidant activity. In this study, allantoin content was determined in concentrations up to 16.96 mg/g. Water extract had the highest content of allantoin while with an increase of ethanol percentage, allantoin content decreased. According to the European Union herbal monograph, *Symphytum officinale* L., radix herbal preparation should be prepared using ethanol 65% (*V/V*) as extraction solvent. Conversely, 65% ethanolic extract allantoin content was under the limit of detection indicating a deviation from traditional expectations. Since the used colorimetric method is robust, this was re-evaluated by LC-Ms analysis where founded results were confirmed.

Table 2: Antioxidant activity and allantoin content of comfrey root extracts

Extract	%EtOH	% DPPH	Allantoin content (mg/g)
1.	65.00	59.96 ±0.45	0
2.	45.00	92.89 ±0.05	7.17 ±0.25
3.	25.00	91.35 ±0.50	12.90 ±1.03
4.	5.00	77.32 ±0.24	14.87 ±2.00
5.	0.00	30.39 ±3.28	16.96 ±0.55

Since 65% ethanolic extract had very low allantoin content (under the limit of detection) this extract was further evaluated for volatile organic compounds. Using gas chromatography coupled with mass spectrometry, a total of 21 compounds were identified (Figure 1): beta-d-Lyxofuranoside, O-nonyl-, C-methyl-scyllo-inositol and lanosterol most abundant ones (Table 3).

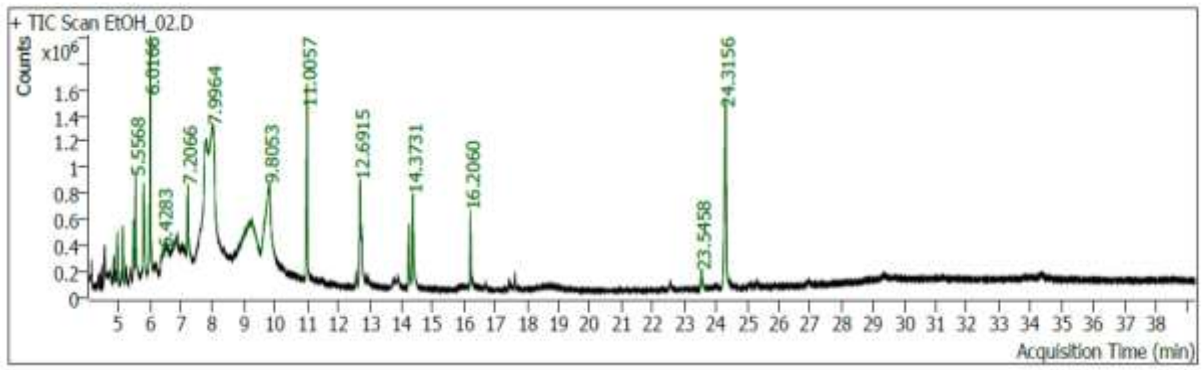


Figure 1: GC/MS chromatogram of 65% ethanolic extract of comfrey root

The largest group were **heterocyclic compounds (**Table 3**)**: Indolizine, Pyrimidine, 6-oxo-5-acetyl-4-hydroxy-1,6-dihydro-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl and 4-Hydroxy-2-mercapto pteridine, followed by **fatty acids and derivatives**: n-Hexadecanoic acid, 9,12-Octadecadienoic acid (Z,Z)- and Glycerol-1-monostearate, **Alcohols and derivatives**: 1,3-Dibutoxy-2-propanol, Scyllo-Inositol, 1-C-methyl- and Helindicine, **Carboxylic acids and derivatives**: Carbamic acid, 1,3,5-Pentanetricarboxylic acid and 3-Deoxy-d-mannoic lactone. From the detected compound two 2,5-Dimethylfuran-3,4(2H,5H)-dione and 5-Hydroxymethylfurfural) were **furans and furan derivatives.** Phenols are compounds that have a hydroxyl group (-OH) directly attached to an aromatic hydrocarbon group. The compounds categorized as **phenols and their derivatives** are: Catechol and 4-Vinylbenzene-1,2-diol**. Sterols and triterpenoids** are large, complex molecules that play vital roles in cell membrane structure and function. The compounds detected in comfrey, included in this category are: beta-Sitosterol **and** Lanosterol. Other detected groups were **Thiols** (tert-Nonyl mercaptan) and **sugars and sugar derivatives (**beta-d-Lyxofuranoside, O-nonyl-).

Table 3. GC-MS profile of comfrey root extract

No.	RT	Compound Name	Formula	%
1.	4.9823	2,5-Dimethylfuran-3,4(2H,5H)-dione	C6H8O3	1.51
2.	5.1465	Indolizine	C8H7N	1.87
3.	5.4903	Carbamic acid		1.41
4.	5.5568	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	C6H8O4	1.66
5.	5.8114	Catechol	C6H6O2	3.74
6.	6.0166	5-Hydroxymethylfurfural	C6H6O3	6.65
7.	6.4283	tert-Nonyl mercaptan	C9H2OS	0.85
8.	6.8231	1,3,5-Pentanetricarboxylic acid	C8H12O6	0.96
9.	7.2066	4-Vinylbenzene-1,2-diol	C8H8O2	2.78
10.	7.7869	Pyrimidine, 6-oxo-5-acetyl-4-hydroxy-1,6-dihydro-	C6H6N2O3	8.43
11.	7.8548	1,3-Dibutoxy-2-propanol	C11H24O3	1.88
12.	7.9964	beta-d-Lyxofuranoside, O-nonyl-	C14H28O5	16.43
13.	9.2334	3-Deoxy-d-mannoic lactone	C6H10O5	8.78
14.	9.8053	Scyllo-Inositol, 1-C-methyl-	C7H14O6	12.13
15.	11.0057	n-Hexadecanoic acid	C16H32O2	6.03
16.	12.6915	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	3.40
17.	12.7283	beta-Sitosterol	C29H50O	2.3
18.	14.3731	4-Hydroxy-2-mercapto pteridine	C6H4N4OS	3.84
19.	16.2060	Glycerol-1-monostearate	C21H42O4	1.64
20.	23.5458	Helindicine	C15H23NO4	0.98
21.	24.3156	Lanosterol	C30H50O	11.83

beta-d-Lyxofuranoside O-nonyl was the dominant compound in 65% ethanolic extract. It possesses several bioactivities. It is a 7-beta-hydroxysteroid dehydrogenase inhibitor, Antiamyloid-Beta, Anti-TGF-Beta, Beta-2-Receptor-Agonist, Beta-Adrenergic receptor blocker, Beta Galactosidase inhibitor, Beta-Glucuronidase inhibitor and Aldehyde oxidase inhibitor (Perumal et al., 2021). The Aldehyde oxidase inhibitor role is vital for cosmetic formulations where those compounds oxidize electrophilic carbons of azaheterocycles, such as pyridine, pyrimidine, and pyridazine and increase solubility, decrease log P, and help to avoid cytochrome P450–mediated metabolism.

CONCLUSION

The evidence from this study suggests that the 65% ethanolic extract prepared according to European Union herbal monograph standards has low levels of allantoin, indicating a deviation from traditional expectations while at the same time, the extract demonstrated a wide range of volatile organic compounds and high antiradical activity, suggesting potential therapeutic benefits beyond its traditional uses. Therefore, future studies on the current topic are required to investigate other polar and non-polar compounds in comfrey root to broaden the medicinal applications of this genus to encompass new pharmacological uses.

REFERENCES

Araujo, L. U., Reis, P. G., Barbosa, L. C. O., 2012. In vivo wound healing effects of *Symphytum officinale* L. leaves extract in different topical formulations. Pharmazie, 67: 355–360.

Borchers, R. 1977. Allantoin determination. Analytical Biochemistry, 79(1–2): 612-61

Božić, I. 2019. Određivanje antioksidacijske aktivnosti i ispitivanje sposobnosti inhibicije kolinesteraza nehlapljivih izolata korijena gaveza. Diplomski rad. Split: Sveučilište u Splitu.

Chen, S., Shang, H., Yang, J., Li, R., & Wu, H. 2018. Effects of different extraction techniques on physicochemical properties and activities of polysaccharides from comfrey (*Symphytum officinale* L.) root. Industrial Crops and Products, 121, 18–25.

Frost, R., O'Meara, S., & MacPherson, H. 2014. The external use of comfrey: A practitioner survey. Complementary therapies in clinical practice, 20(4), 347-355.

Jakobek, L., Šeruga, M., Novak, I., & Medvidovic-Kosanovic, M. 2007. Flavonols, phenolic acids and antioxidant activity of some red fruits. Deutsche Lebensmittel Rundschau, 103:369–377

Kucera, A., Barna, M., Holcova, S., Horacek, O., Hladiková, M., & Ottillinger, B. 2018. Tolerability and effectiveness of an antitrauma cream with comfrey herb extract in pediatric use with application on intact and on broken skin. International Journal of Pediatrics and Adolescent Medicine, 5(4), 135-141.

Melnyk, N., Popowski, D., Strawa, J. W., Przygodzińska, K., Tomczyk, M., Piwowarski, J. P., & Granica, S. 2024. Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.). Journal of Ethnopharmacology, 10;318, 116968.

Perumal, G., Prabhu, K., Rao, M. R. K., Janaki, C. S., Kalaivannan, J., & Kavimani, M. 2021. The GC MS Analysis of Ethyl Acetate Extract of One Herbal Plant,'Euphorbia Hypericifolia'. Nveo-Natural Volatiles & Essential Oils Journal| NVEO, 4078-4083.

Savić, V. L., Nikolić, V. D., Arsić, I. A., Stanojević, L. P., Najman, S. J., Stojanović, S., & Mladenović-Ranisavljević, I. I. 2015. Comparative study of the biological activity of allantoin and aqueous extract of the comfrey root. Phytotherapy Research, 29(8), 1117-1122.

Smith, D. B., & Jacobson, B. H. 2011. Effect of a blend of comfrey root extract (*Symphytum officinale* L.) and tannic acid creams in the treatment of osteoarthritis of the knee: randomized, placebo-controlled, double-blind, multiclinical trials. Journal of Chiropractic Medicine, 10(3): 147-156.

Staiger, C. 2012. Comfrey: a clinical overview. Phytotherapy Research, 26(10): 1441–1448.

Trifan, A., Opitz, S. E., Josuran, R., Grubelnik, A., Esslinger, N., Peter, S., & Wolfram, E. 2018. Is comfrey root more than toxic pyrrolizidine alkaloids? Salvianolic acids among antioxidant polyphenols in comfrey (*Symphytum officinale* L.) roots. Food and Chemical Toxicology, 112: 178-187.

DESIGN AND APPLICATION OF AN AUTOMATIC PET FEEDER WITH TWO-WAY SPLITTER SUITABLE FOR GRID-CONNECTED OR SOLAR-POWERED USE

Görkem Şen
Electronics and Automation Department, Ipsala Vocational School, Trakya University, Edirne, Türkiye
gorkemsen@trakya.edu.tr

ABSTRACT

Pet owners see their pets as a source of happiness. When they take care of their pets, they get rid of stress and start to feel better. Besides that, when they adopt a cat or a dog, pet owners take on various responsibilities, including feeding, sheltering, and regular veterinary check-ups. Pet owners like to take care of their pets by feeding them on time. However, the real challenge for most pet owners is feeding their pets when they are away from home, such as busy work schedules or business trips. For this reason, pet owners need an automatic pet feeder to solve the problem of feeding their pets. Especially pet owners who have more than one pet need the automatic pet feeder with high dry food capacity and the ability to dispense equal amounts of food. In this study, the automatic pet feeder designed to feed two dogs at the same time, which can be operated with both the solar energy and the power grid, is performed. The automatic pet feeder with two-way splitter basically consists of an ac-dc converter, 12 Volt photovoltaic (PV) panel, a solar battery charge controller, a battery group, digital timers, a DC motor, a DC motor controller, a sound system, 19 liter dry food reservoir and a food portion dispenser. If mentioned system is operated with the power grid, the ac-dc converter is used, but if it is operated with the solar energy, 12 Volt PV panel is used. With the design, the automatic pet feeder can be positioned both inside the house and in the garden. While it can be operated with the power grid when used indoors, it can be powered either by the solar energy or the power grid when used in the garden. Also, thanks to the battery group inside the automatic pet feeder, it is not affected by power outages or periods of time when the sun is not shining. The designed automatic pet feeder can be operated repeatedly in different time periods, 24 hours a day, 7 days a week, with digital timers. In addition, the amount of dry food to be given to pets at each meal can be easily adjusted by determining the rotation speed and rotation time of the food portion dispenser. Owing to its design, equal amounts of food are given to two dogs at the same time. Also, at each meal, the dogs are notified that it is time to eat with the music given by the sound system in the automatic pet feeder. As a result, this designed and implemented automatic pet feeder provides great convenience to pet owners as has a high dry food capacity, has a two-way splitter, can operated with both the solar energy and the power grid, and can easily adjust the number of meals and the amount of food. When pet owners are away from home, they do not need to ask anyone else for help to feed their pets by using this automatic pet feeder. In addition, pets are fed regularly.

Keywords: Automatic pet feeder, Grid-connected system, Off-grid solar system, Renewable energy

INTRODUCTION

The bond that humans establish with animals dates back to ancient times. Researches show that animals began to be domesticated by humans approximately 12 thousand years ago. Today, many people continue to share their homes, gardens with one or more pets. A pet is often considered a member of the family. Dogs and cats are the most popular pets (Sangvanloy and Sookhanaphibarn, 2020). Taking care of a pet affects people positively both emotionally, psychologically and physically (Liu, 2021). Studies have shown that people who care for pets can keep their blood pressure low and their heart rate controllable under stressful conditions. Therefore, owning a pet has been shown to reduce the change of heart disease and also reduce stress, thus improving performance. (Bembde et al., 2023).

One of the greatest happiness in human life is owning a pet. Some of the people who do not own pet also aim to have pet in the future. However, pets need to be cared for properly. When people adopt a cat or a dog, they take on various responsibilities, including feeding, sheltering, and regular veterinary check-ups. Since they are members of our family, it is an important task to feed them on time. To raise a healthy pet, each pet's food and feeding habits should be carefully examined. When pets are not fed on time, their health is directly affected (Vrishanka et al., 2021). Pet owners like to take care of their pets by feeding them on time. But nowadays, pet owners often face the situation of being away from home for long periods of time, such as busy work schedules or business trips, which causes pets not to be fed on time, irregular feeding, hungry etc. (Liu, 2021). For this reason, pet owners need an automatic pet feeder to solve the problem of feeding their pets.

One of the new approaches in pet feeding is automatic pet feeders. These devices will help pet owners take care of their pets. Automatic pet feeders can feed pets even if their owners are not at home. However, as the number of pets cared for in a household increases, automatic pet feeders on the current market may become insufficient. Both food capacity and providing equal amounts of meals emerge as problems. Additionally, pet owners can care for their pets not only at home but also in the garden. Therefore, automatic pet feeders must also be resistant to the external environment. In addition, people who care for their pets in the garden or in a rural area where there is no electricity need automatic pet feeders that can also work with solar energy, as they are environmentally friendly and can work independently of the power grid. Using this type of automatic pet feeders will help reduce greenhouse gas emissions and eliminate feeding irregularities of pets that may occur due to power outages in the power grid.

Especially in Türkiye, it is possible to use automatic pet feeders powered by solar energy. Türkiye is suitable for the use of solar energy since it is located between the 26th and 45th east meridians and the 36th and 42nd northern parallels (Kotcioğlu, 2011). Daily sunlight duration is nearly 5 hours in winter, 7 hours in spring, and 11 hours in summer months in Türkiye (Yüksel and Türkboyları, 2018). Therefore, Türkiye has a notable potential of solar energy and this potential is shown in Figure 1 (Kabalci et al., 2016). Colors of atlas show values of total annually average and solar energy potential is directly proportionate to region and notable amount of power could be acquired from solar energy in Türkiye.

Figure 1. Solar energy potential atlas of Türkiye (Kabalci et al., 2016).

In this study, the automatic pet feeder designed to feed two dogs at the same time, which can be operated with both the solar energy and the power grid, is performed. The automatic pet feeder with two-way splitter basically consists of an AC-DC converter, 12 Volt photovoltaic (PV) panel, a solar battery charge controller, a battery group, digital timers, a DC motor, a DC motor controller, a sound system, 19 liter dry food reservoir and a food portion dispenser. If mentioned system is operated with the power grid, the AC-DC converter is used, but if it is operated with the solar energy, 12 Volt PV panel is used. Thanks to the battery group inside the automatic pet feeder, it is not affected by power outages or periods of time when the sun is not shining. The designed automatic pet feeder can be operated repeatedly in different time periods, 24 hours a day, 7 days a week, with digital timers or manually. Thanks to its design, equal amounts of food are given to two dogs at the same time, accompanied by music.

MATERIAL AND METHOD

Study Area

Çanakkale is in the northwest of Türkiye and is located within the Marmara Region. Çanakkale has 12 districts and one of them is Ezine. Ezine's location is shown in Figure 2.

Figure 2. Ezine’s location in Çanakkale, Türkiye where the study is carried out

This study is realized in a detached house with a garden in Ezine. The feeding of two adult dogs kept in the garden is provided by the automatic pet feeder introduced in this study.

DESIGN AND REALIZATION OF THE PROPOSED SYSTEM

In this study, thanks to the two-way splitter structure of the proposed automatic pet feeder system, two dogs can be fed equal amounts of food every day. Additionally, depending on user preference, the proposed system can work with either the power grid or the solar energy.

Grid-connected Operation of the Proposed System

The grid-connected operation scheme of the proposed system is shown in Figure 3. As seen in Figure 3, the battery group is charged through the AC-DC converter and the charge control unit connected to the power grid. The battery group powers the control unit, and the control unit provides energy flow to the dc motor that rotates the food portion dispenser, either time-adjusted or manually. Additionally, the sound system plays music when the food is given.

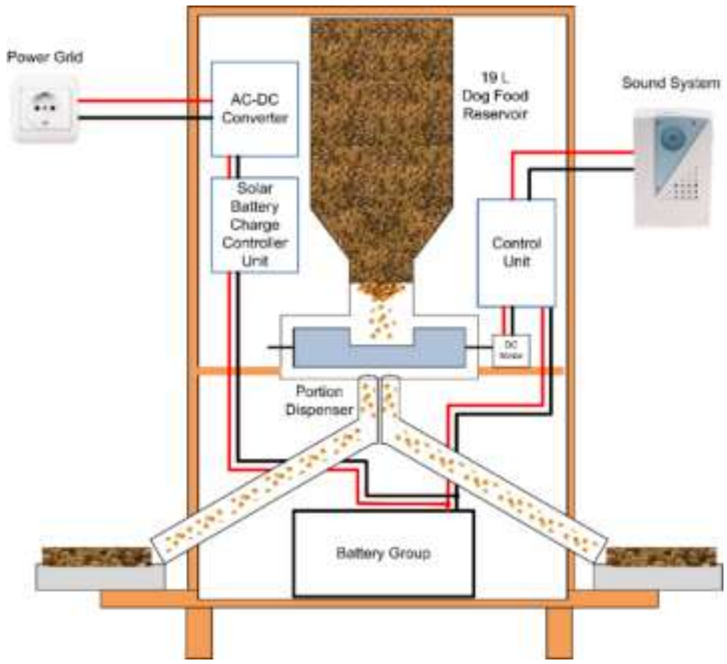


Figure 3. Grid-connected operation scheme of the proposed system

Off-grid Solar-powered Operation of the Proposed System

The off-grid solar-powered operation scheme of the proposed system is shown in Figure 4. As seen in Figure 4, PV panel charges the battery group with the solar charge controller. The battery group powers the control unit. The control unit provides energy flow to the DC motor that rotates the food portion dispenser, either time-adjusted or manually. The sound system plays music when the food is given.

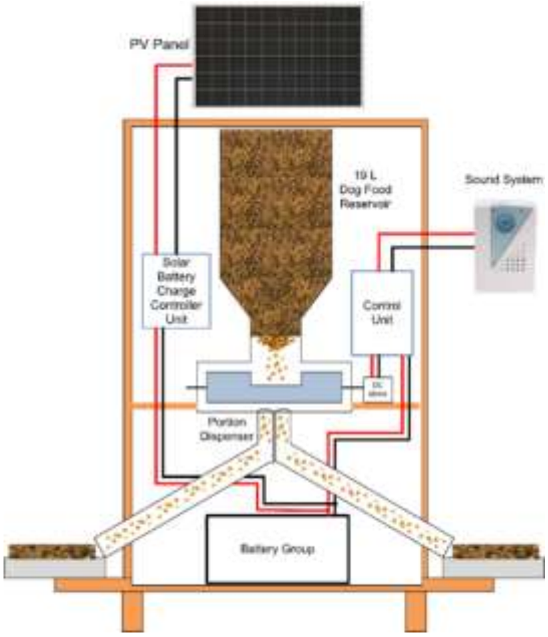


Figure 4. Off-grid solar-powered operation scheme of the proposed system

General Structure and Operation of the Proposed System

A photograph of the automatic pet feeder with two-way splitter is shown in Figure 5. The automatic pet feeder with two-way splitter basically consists of the energy source (power grid or 12 Volt PV panel), the AC-DC converter (only grid-connected operation mode), the solar battery charge controller, the battery group, digital timers, the DC motor, the DC motor controller, the sound system, 19 liter dry food reservoir and the food portion dispenser.

Figure 5. Photograph of the proposed automatic pet feeder

Depending on the user's preference, either the power grid or 12 Volt PV panel is used as the energy source in this study. The voltage value of the electricity used in houses in Türkiye is 220 Volt (rms value) and the frequency is 50 Hertz (Hz). One PV panel is preferred to be used in this study. This PV panel is rated at 12 Volt, 170 Watt. Characteristics of PV panel are expressed in Table 1.

Table 1. Characteristics of PV panel

Name	Rating
Panel Maximum Power	205 W
Peak Voltage	21.45V
Peak Current	9.6 A
Open Circuit Voltage	25.92 V
Short Circuit Current	9.9 A

The introduced system includes the AC-DC converter that is used only in grid-connected operation. The input voltage is 220 Volt AC and the output voltage is 13.8 Volt DC and output current 3,5 A DC.

The solar charge controller is used to regulate the flow of voltage and current from the PV panel to the battery group so that it can protect the battery group from damage due to overcharging. Some characteristics of the solar battery charge controller are shown in Table 2.

Table 2. Some characteristics of solar battery charge controller

Name	**Rating**
Rated Charge Current	10 A
Operating Voltage	12/24Volt auto sensing/auto switching
Control Method	Pulse width modulation (PWM) pulse-duration modulation charge mode
Working Temperature	-35ºC to +60ºC

Another important unit is the battery group. Batteries convert electrical energy into chemical energy for energy storage. In this study, the battery group consists of 6 valve-regulated rechargeable batteries connected in parallel. The capacity of each valve regulated rechargeable battery is 12 Ah, 12 Volt. Battery group of the automatic pet feeder is shown in Figure 6.

Figure 6. Battery group of the automatic pet feeder in use

An electric motor takes electrical energy and produces mechanical energy. Electric motors come in various ratings and sizes. In this study, 12 Volt DC motor is used to rotate the food portion dispenser. The specifications of the DC motor used are shown in Table 3.

Table 3. Characteristics of DC motor

Name	**Rating**
Operating Voltage	12 - 14,4 Volt
Speed	20000 RPM
Power	20 Watt

In the proposed automatic pet feeder, a sound system is used to notify dogs that it is time to eat. Photographs of the sound system are shown in Figure 7. In the construction of this sound system, a wireless doorbell with 24 melodies, a relay and a button are used. The wireless doorbell circuit is modified and transformed into a wired structure that works with a trigger signal. When the DC motor starts, the trigger signal comes to the sound system and the selected music plays. In addition, thanks to the added button, the selected music can be played manually at any time.

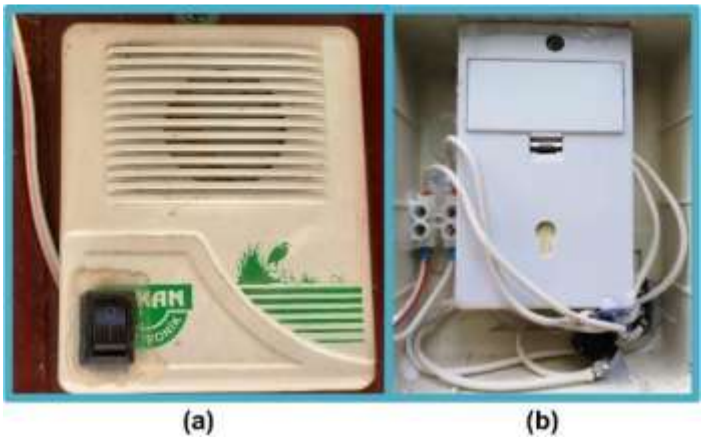


Figure 7. Photographs of the sound system in use

The parts of the automatic pet feeder related to food are the 19 liter dry food reservoir, the two-way splitter and the food portion dispenser. These parts are shown in Figure 8. The 19 liter dry food reservoir is made of a 19 liter plastic water bottle. The food portion dispenser mechanism is made of plastic water pipes of different sizes. The structure seen in Figure 8.b is connected to the shaft of the DC motor. When the shaft of the DC motor rotates, the food portion dispenser also rotates. As a result of this rotation, the dry food is put to the food bowls via two different paths by the two-way splitter.

Figure 8. (a) Photograph of two-way splitter, (b) Photograph of food portion dispenser
Proposed system's electrical connection and control unit are shown in Figure 9.

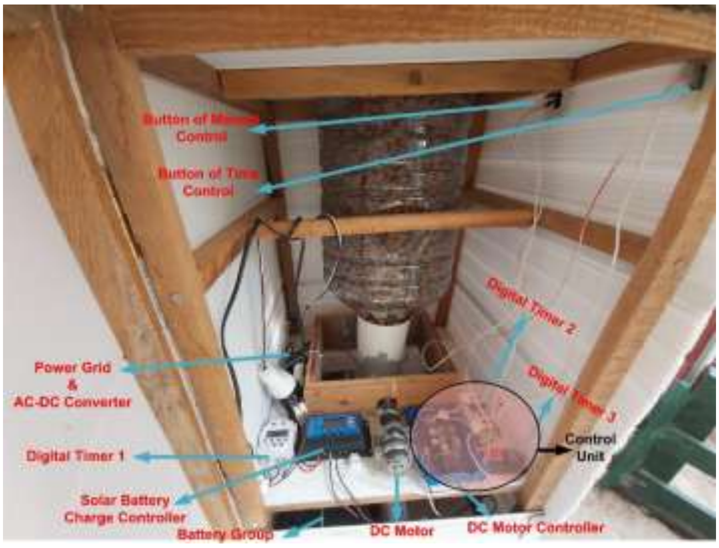


Figure 9. Photograph of proposed system's electrical connection and control unit

The operating steps of the proposed automatic pet feeder are as follows:

- The battery group is connected to the solar battery charge controller and the necessary settings of charge controller are made.
- In grid-connected operation, 220 Volt power grid voltage is converted to 13.8 Volt DC via the AC-DC converter. Then, the output of the AC-DC converter is connected to the solar battery charge controller via Digital Timer 1 (AC Type). Digital Timer 1 is used to set the time periods in which the battery group will be charged. Otherwise, the batteries will always be subject to charging.
- In off-grid solar-powered operation, PV panel terminals are directly connected to the solar battery charge controller. The AC-DC converter and Digital Timer 1 are not used in this operation.
- Terminals of the battery group are connected to the control unit. The control unit controls the operations of the DC motor and the sound system. The control unit consists of 5 elements. These are Digital Timer 2 (DC Type), Digital Timer 3 (DC Type), the DC motor controller, button of manual control and button of time control. When the button of manual control is pressed, equal amounts of food are given to both dog bowls. The button of time control turns the time adjusted operation on and off. A trigger signal is generated 24/7 with Digital Timer 2. Digital Timer 3 operates according to the trigger signal generated by Digital Timer 2 and determines how many seconds the DC motor will operate after the trigger signal arrives. The output of Digital Timer 3 is connected to the DC motor controller. The speed of the DC motor is adjusted with the DC motor controller. The amount of food to be given in each meal depends on the time set in Digital Timer 3 and the speed setting made in the DC motor controller.
- As soon as the DC motor rotates, a trigger signal is sent to the sound system and the previously specified music starts playing.
- With the rotation of the DC motor, the food portion dispenser starts to rotate and delivers equal amounts of food to the two dog food bowls in the specified time

As a result, the energy needs of the automatic pet feeder can be supplied from the power grid or the PV panel. Moreover, the two-way splitter structure works successfully. Thus, it can give equal amounts of food arranged in two food bowls at each meal, both time adjusted and manually.

CONCLUSIONS

The bond between humans and dogs dates back to a very distant past, and there are various positive effects that owning a dog brings to human life, such as relief from stress, security. However, pet owners undertake various responsibilities, such as regular feeding. Due to today's business life, it may not be possible to feed the dog regularly. Dogs are also negatively affected by this situation. To eliminate this negative situation, pet owners need automatic pet feeders.

In this study, the automatic pet feeder with the two-way splitter and powered by either solar energy or the power grid is introduced. This study is carried out in the detached house with a garden located in Ezine, Çanakkale, Türkiye. Since there is 19 liter dry food reservoir in the proposed system, the food needs of two dogs can be met with equal meals for days. Thanks to the battery group within the system, the feeding needs of the animals can be met 24 hours a day, 7 days a week. Successful results have been achieved using this proposed automatic pet feeder. Since dogs were fed regular and equal meals, their weight remained stable and they did not experience stress and unhappiness. Additionally, pet owners are able to continue their daily lives comfortably.

As a result, the energy needs of the pet feeder can be provided from the power grid or PV panel according to the user's preference. Additionally, the two-way splitter structure works successfully. Thus, it can give equal amounts of food arranged in two food bowls at each meal. Based on this system, designs can be created to feed more pets. By using solar energy in this study, it not only contributes to the reduction of greenhouse gas emissions but also enables the use of automatic pet feeders in places where there is no electricity.

REFERENCES

Sangvanloy T., Sookhanaphibarn K. 2020. Automatic Pet Food Dispenser by Using Internet of Things (IoT). 2020 IEEE 2nd Global Conference on Life Sciences and Technologies (LifeTech 2020). 10-12 March 2020. Kyoto, Japan. 132.

Liu R. 2021. Automatic Pet Feeder based on Single Chip Microcomputer. Journal of Physics Conference Series. 2037(1):012104.

Bembde M., Ranjan N. M., Kamble P., Chavan A., Yelmar A., Mane R. 2023.Robotic Day-Care for Pets using Sensors and Raspberry Pi. 2023 International Conference on Sustainable Computing and Smart Systems (ICSCSS). 14-16 June 2023. Coimbatore, India. 994.

Vrishanka P. N., Prabhakar P., Shet D., Rupali K. 2021. Automated Pet Feeder using IoT. 2021 IEEE International Conference on Mobile Networks and Wireless Communications (ICMNWC). 03-04 December 2021. Tumkur, Karnataka, India.

Kotcioğlu I. 2011. Clean and Sustainable Energy Policies in Turkey. Renewable and Sustainable Energy Reviews. 15(9): 5111-5119.

Yüksel A. N., Türkboyları E. Y. 2018. Use Of Solar Panels in Greenhouse Soil Disinfection. International Advanced Researches and Engineering Journal. 02(02): 195-199.

Kabalci Y., Kabalci E., Canbaz R., Calpbinici A. 2016. Design and Implementation of a Solar Plant and Irrigation System with Remote Monitoring and Remote Control Infrastructures. Solar Energy. 139 (2016): 506–517.

DESIGN AND IMPLEMENTATION OF A SOLAR-BASED AUTOMATIC PET FEEDER AND WATER DISPENSER

Görkem Şen

Electronics and Automation Department, Ipsala Vocational School, Trakya University, Edirne, Türkiye

gorkemsen@trakya.edu.tr

ABSTRACT

Nowadays, most people keep pets in their homes or gardens to reduce the negative effects of daily life such as loneliness, stress, boredom and unhappiness. Especially with the pandemic period, there has been an increase in the number of people taking care of pets. Pet owners assume very serious responsibilities. The most important of these responsibilities are to feed pets regularly and meet their water needs. In order for pets to live a healthy and long life, they need to have a regular eating and drinking schedule like humans. However, no matter how much pet owners enjoy taking care of their pets, they may not be able to feed their pets regularly due to some reasons, such as today's busy work schedule or business travel. To eliminate this problem, pet owners need automatic pet feeders and water dispenser systems. Additionally, people who care for their pets in the gardens or in rural areas where there is no electricity, want to use automatic pet feeders and water dispensers powered by renewable energy. In this study, an off-grid solar-based automatic pet feeder and water dispenser that can provide dry food and water is introduced. The offered system has two main functions, namely feeding and water giving, and these functions can operate both manually and automatically in various time periods, 24 hours a day, 7 days a week. The automatic pet feeder and water dispenser basically consists of 12 Volt photovoltaic (PV) panel, a solar battery charge controller, a battery group, digital timers, a DC motor, a DC motor controller, a sound system, 19 liter dry food reservoir, a food portion dispenser, a water level control circuit, a solenoid valve and 38 liter water tank. The water dispenser is always active. The water level in the water bowl is detected through minimum and maximum water level sensors. When the water level drops to the minimum, water flow is provided from the 38 liter water tank to the water bowl through the solenoid valve until the water level reaches the maximum. Additionally, the pet feeder can provide equal portions of dry food at different times of the day. Each pet's feeding amount is different. For this reason, the rotation speed and rotation time of the food dispenser in the automatic pet feeder can be easily adjusted, allowing pets to be fed with the ideal amount of food. Furthermore, the automatic pet feeder provides music during each meal, signaling to the pet that it is time to eat. As a result, this introduced system which works with renewable energy, provides serious convenience to pet owners due to its features such as having high dry food and water capacities, adjusting the portion size of meals, providing regular pet feeding, and not being affected by power outages. By using the proposed automatic pet feeder and water dispenser, there will be no need to leave pets elsewhere to be fed and to ask for help from someone else.

Keywords: Automatic pet feeder, Automatic water dispenser, Off-grid solar system, Renewable energy

INTRODUCTION

Pets are tamed and fed by humans to turn them into animals with pleasant personalities. Pets that people keep most are dogs and cats. According to archaeological research, the first domesticated animals are thought to be dogs approximately 12,000 years ago (Harahap et al., 2022). Dogs are the most beloved pets in history and are one of the most common pets owned by people today, according to a recent study (Boateng et al., 2022).

Nowadays, many people share their homes and gardens with one or more dogs. Especially during the pandemic period, there was an increase in the number of people adopting dogs. Additionally, in today's generation, there are a significant number of people who want to own a dog (Koley et al., 2020). Currently, dogs are not only guardian animals, but also one of the owner's family members (Vania et al., 2016). By incorporating dogs into people's daily life routines, they provide physical, emotional and psychological benefits to people (Liu, 2021). Owning a dog meets a person's social needs and provides many other benefits, such as stress relief, boredom relief and responsibility training (Airikala et al., 2020). Additionally, some studies have shown that dogs can help treat children with developmental disorders such as autism (Harahap et al., 2022).

Even though owning a dog is one of the greatest joys in human life, it is not easy to raise a dog in practice because dogs need various care to stay healthy and happy (Harahap et al., 2022). Pet owners take on very serious responsibilities such as feeding, sheltering and regular veterinary check-ups. The most important of these responsibilities is to regularly feed the pets and meet their water needs (Koley et al., 2020). Since they are members of our family, feeding them on time and meeting their water needs is an important task. To raise a healthy dog, each pet's eating habits should be carefully examined. When pets are not fed on time, their health is directly affected (Vrishanka et al., 2021). However, no matter how much people love their pets, they may neglect to care for their pets in terms of providing food and water at equal intervals due to busy working lives, business trips or emergencies (Koley et al., 2020). For example, during the pandemic period, some pet owners were unable to care for their dogs because they were receiving health care in the hospital. As a result, it was observed that some dogs' reactions to their owners changed, and some dogs developed malnutrition. In some cases, dogs even died of starvation (Harahap et al., 2022). Therefore, pet owners need an automatic pet feeder to solve their pets' feeding problem.

Automatic pet feeders are devices that both save time for pet owners and ensure regular feeding of pets (Boateng et al., 2022). Automatic pet feeders can feed pets even if their owners are not at home. However, current automatic pet feeders on the market cannot meet the needs of every pet owner. It may be inadequate both in terms of food capacity and water capacity. In addition, pet owners can take care of their pets not only at home but also in the garden. Therefore, automatic pet feeders must also be resistant to the external environment. It is also important to meet the needs of pet owners in rural areas where there is no electricity. Hence, there is a need for solar-powered automatic pet feeders and water dispensers. The use of this type of automatic pet feeders and water dispensers will help reduce greenhouse gas emissions and eliminate irregularities in the feeding of pets due to power outages in the power grid.

Türkiye is suitable for the use of solar energy because it is located between the 26th and 45th eastern meridians and the 36th and 42nd northern parallels (Kotcioğlu, 2011). Daily sunlight duration in Türkiye is approximately 5 hours in winter, 7 hours in spring, and 11 hours in summer (Yüksel and Türkboyları, 2018). Therefore, Türkiye has an important solar energy potential and this potential is shown in Figure 1 (Kabalci et al., 2016). So, it is possible to use solar-powered automatic pet feeders and water dispensers in Türkiye.

Figure 1. Türkiye's solar energy potential atlas (Kabalci et al., 2016).

In this study, the off-grid solar-based automatic pet feeder and water dispenser that can provide dry food and water is introduced. The offered system has two main functions, namely feeding and water giving, and these functions can operate both manually and automatically in various time periods, 24 hours a day, 7 days a week. The automatic pet feeder and water dispenser basically consists of 12 Volt photovoltaic (PV) panel, a solar battery charge controller, a battery group, digital timers, a DC motor, a DC motor controller, a sound system, 19 liter dry food reservoir, a food portion dispenser, a water level control circuit, a solenoid valve and 38 liter water tank. This introduced system which works with renewable energy, provides serious convenience to pet owners due to its features such as having high dry food and water capacities, adjusting the portion size of meals, providing regular pet feeding, and not being affected by power outages.

MATERIAL AND METHOD

Study Area

Located within the borders of the Marmara Region, Çanakkale is located in the northwest of Türkiye. In addition, just like Istanbul, it has lands on both Asian and European continents. Neighboring provinces of Çanakkale are Edirne, Tekirdağ and Balıkesir. Çanakkale has 12 districts and one of these districts is Ezine, whose location is shown in Figure 2. This study is realized in an approximately three-acre olive grove in Ezine. The regular food and water needs of a dog living in this agricultural estate where there is no power grid are met by the solar-powered automatic pet feeder and water dispenser introduced in this study.

Figure 2. The location of Ezine, Çanakkale in Türkiye where the study is carried out

DESIGN AND REALIZATION OF THE PROPOSED SYSTEM

In this study, the automatic pet feeder and water dispenser that works with solar energy, independent of the power grid, and meets a dog's regular food and water needs is made.

Structure of the Automatic Water Dispenser

The general scheme and photographs of the automatic water dispenser part of the proposed system is shown in Figure 3 and Figure 4, respectively.

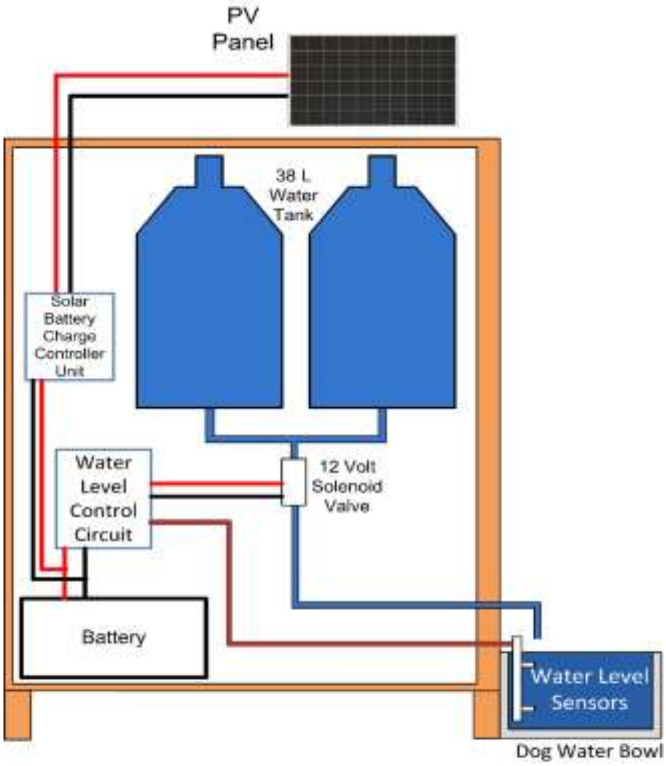


Figure 3. Off-grid solar-powered operation scheme of the automatic water dispenser

Figure 4. Photographs of the proposed automatic water dispenser

As seen in Figure 3, the structure of the automatic water dispenser consists of 12 Volt PV panel, the solar battery charge controller, the battery group, 38 liter water tank, the solenoid valve, water level sensors, the water level control circuit and dog water bowl.

Structure of the Automatic Pet Feeder

The general scheme and photographs of the automatic pet feeder part of the proposed system is shown in Figure 5 and Figure 6, respectively.

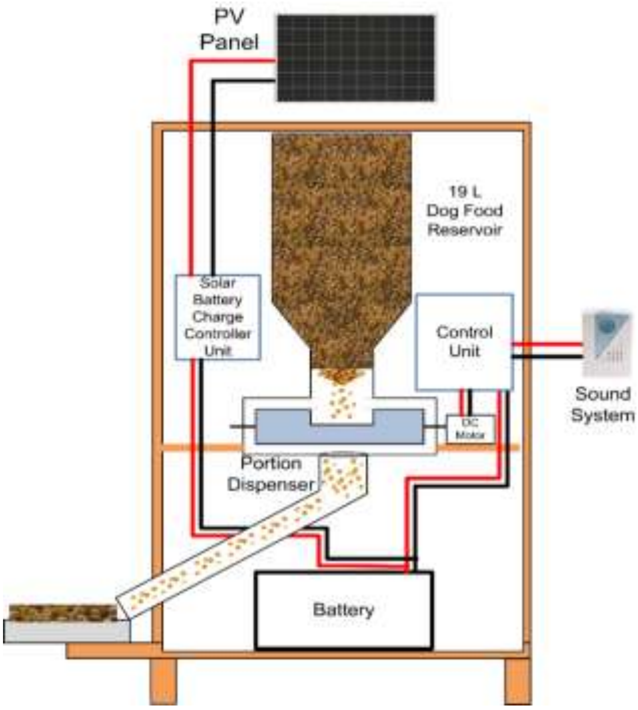


Figure 5. Off-grid solar-powered operation scheme of the automatic pet feeder

Figure 6. Photographs of the proposed automatic pet feeder

As seen in Figure 5, the structure of the automatic pet feeder consists of 12 Volt PV panel, the solar battery charge controller, the battery group, digital timers, the DC motor, the DC motor controller, the sound system, 19 liter dry food reservoir and the food portion dispenser.

General Structure and Operation of the Proposed System

Overall scheme of the proposed system is shown in Figure 7 and photograph of the proposed system is demonstrated in Figure 8, respectively.

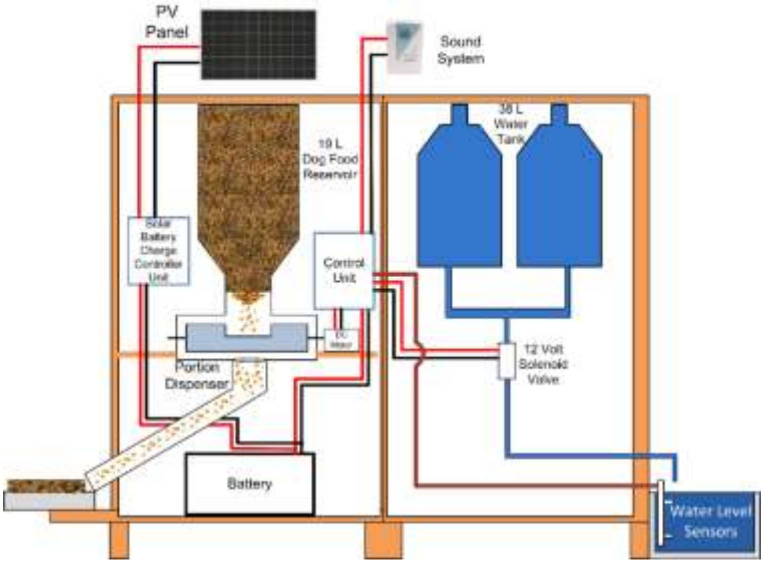


Figure 7. Overall scheme of the proposed system

Figure 8. Photograph of the proposed automatic pet feeder and water dispenser

As seen in Figure 7 and Figure 8, the introduced system consists of 2 parts that perform 2 separate functions. These are feeding and water giving, and these functions can operate both manually and automatically in various time periods, 24 hours a day, 7 days a week.

In this study, 12 Volt, 170 Watt PV panel is used. Characteristics of PV panel are presented in Table 1.

Table 1. Characteristics of PV panel

Name	Rating
Panel Maximum Power	205 W
Peak Voltage	21.45V
Peak Current	9.6 A
Open Circuit Voltage	25.92 V
Short Circuit Current	9.9 A

The solar charge controller is used to regulate the flow of current and voltage from PV panel to the battery group. Thus, it can protect the battery from damage due to overcharging. Some characteristics of the solar battery charge controller are presented in Table 2.

Table 2. Some characteristics of solar battery charge controller

Name	Rating
Rated Charge Current	20 A
Operating Voltage	12/24Volt auto sensing/auto switching
Control Method	Pulse width modulation (PWM) pulse-duration modulation charge mode
Working Temperature	-35ºC to +60ºC

The battery group is very important in solar-powered systems. In this study, the battery group consists of 3 deep cycle gel batteries connected in parallel. The capacity of each gel battery is 12Volt, 100Ah. Battery group of the automatic pet feeder is shown in Figure 9.

Figure 9. The battery group of the solar-based automatic pet feeder and water dispenser in use

In this study, 12 Volt DC motor is used and the task of this motor is to rotate the food portion dispenser. The characteristics of the DC motor are presented in Table 3.

Table 3. Characteristics of DC motor

Name	Rating
Operating Voltage	12 - 14,4 Volt
Speed	20000 RPM
Power	20 Watt

In the automatic pet feeder and water dispenser, the sound system is used to inform the dog that it is time to eat. Photographs of the sound system are shown in Figure 10. In the construction of mentioned sound system, a wireless doorbell with 24 melodies, a relay and a button are used. The wireless doorbell circuit is modified and transformed into a wired structure. Thus, the sound system works with a trigger signal. When the DC motor starts, the trigger signal comes to the sound system and the chosen music plays. In addition, thanks to the added button, the chosen music can be played manually at any time.

(a) (b)

Figure 10. Photographs of the sound system in use

The parts of the proposed system related to food are 19 liter dry food reservoir, the one-way splitter and the food portion dispenser. These parts are shown in Figure 11. The dry food reservoir is made of 19 liter plastic water bottle. The food portion dispenser mechanism is made of plastic water pipes of different sizes. The structure seen in Figure 11.b is connected to the shaft of the DC motor. When the shaft of the DC motor rotates, the food portion dispenser also rotates. As a result of this rotation, the dry food is put to the dog food bowl.

(a) (b)

Figure 11. (a) Photograph of one-way splitter, (b) Photograph of food dispenser

The parts of the proposed system related to water are 38 liter water tank, 12 Volt solenoid valve, water level sensors, dog water bowl. These parts are shown in Figure 12.

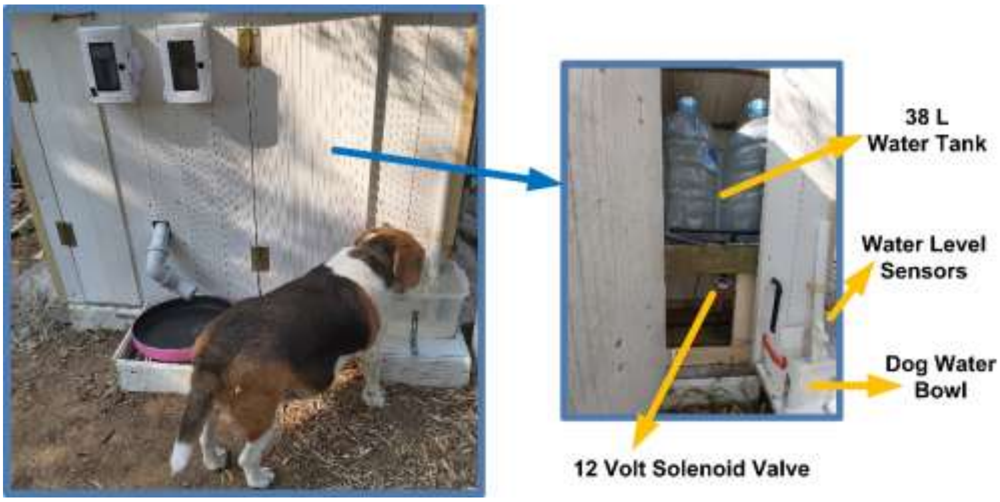


Figure 12. Photograph of the parts of the proposed system related to water

The control unit controls operations of the DC motor, the solenoid valve and the sound system. The control unit consists of 7 elements. These are Digital Timer 1, Digital Timer 2, the DC motor controller, the button of manual control, the button of time control, the button of manual water supply and the water level control circuit. The button of time control turns the time adjusted operation on and off. The pet feeder can give food at any time with the button of manual control. Also, water can be poured into the dog water bowl at any time with the button of manual water supply. The control unit is shown in Figure 13.

Figure 13. Control unit of the automatic pet feeder and water dispenser

The operating steps of the proposed automatic pet feeder and water dispenser are as follows:

- The battery group is connected to the solar battery charge controller. The necessary settings of charge controller are made.
- PV panel terminals are directly connected to the solar battery charge controller.
- The battery group is charged with solar energy during the day.
- Terminals of the battery group are connected to the control unit. For operation of the DC motor, trigger signals are generated 24/7 with Digital Timer 1 or button of manual control. Digital Timer 2 operates according to the trigger signal generated by Digital Timer 1 and determines how many seconds the DC motor will operate after the trigger signal arrives. The output of Digital Timer 2 is connected to the DC motor controller. The speed of the DC motor is adjusted with the DC motor controller. The amount of food to be given in each meal depends on the time set in Digital Timer 2 and the speed setting made in the DC motor controller.
- When it's time to eat, the food portion dispenser starts to rotate and delivers dry food to the dog food bowl. As soon as the DC motor rotates, the trigger signal is sent to the sound system and the previously chosen music starts playing.
- Minimum and maximum water level data received from the dog water bowl are sent to the water level control circuit. This circuit provides energy flow from the battery group to the solenoid valve when the water level in the dog water bowl is minimum. As a result, water flows from 38 liter water tank to the dog water bowl. When the water level in the dog water bowl reaches the maximum level, the water level control circuit cuts off the energy of the solenoid valve. Thanks to this mode of operation, water is constantly available within the maximum and minimum water levels set in the dog water bowl. Additionally, water flow into the dog water bowl can be achieved by manually energizing the solenoid valve without using the water level control circuit.

As a result, the energy needs of the automatic pet feeder and water dispenser can be supplied from PV panel. Moreover, the automatic pet feeder with one-way splitter structure and automatic water dispenser work successfully both time adjusted and manually.

CONCLUSIONS

Regular eating food and drinking water is so important for pets like human beings. If pet owners are with their pets, the pets do not have any problems with food and water since the pets are under constant supervision. However, pet owners cannot be with their pets all the time for different reasons. As a result, problems such as irregular nutrition and insufficient water consumption arise. To eliminate these problems, pet owners need automatic pet feeders and water dispensers. Moreover, pet owners are looking for systems powered by renewable energy.

In this study, the automatic pet feeder and water dispenser powered by solar energy is introduced. This study is carried out in an approximately three acres of olive grove in Ezine, Çanakkale, Türkiye. Since there is no power grid in the specified agricultural land, a dog's water and food needs are met regularly with the proposed system. With the using of this system, the pet owner is able to continue his daily life without experiencing stress.

As a result, it is confirmed that the need of energy in the automatic pet feeder and water dispenser can be met from solar energy. The proposed pet feeder is a suitable solution to meet the food and water needs of all pets. In addition, both the food and water capacity of the proposed pet feeder and the PV panel and battery capacity can be easily determined according to the need. Therefore, the use of the proposed pet feeder will be beneficial for both pets and pet owners.

REFERENCES

Harahap R. K., Wibowo E. P., Nur'ainingsih D., Wijaya A. K., Widyastuti, Anindya R. A. S. C. 2022. Dogs Feed Smart System With Food Scales Indicator IoT Based. 4th International Conference on Cybernetics and Intelligent System (ICORIS). 08-09 October 2022. Prapat, Indonesia.

Boateng M. A., Akparibo A. R. 2022. A Multifunctional Automatic Dog-Feeder with Bluetooth and Wi-Fi Connectivity. IEEE 2nd International Conference on Mobile Networks and Wireless Communications (ICMNWC). 02-03 December 2022. Tumkur, Karnataka, India.

Koley S., Srimani S., Nandy D., Pal P., Biswas S., Sarkar I. 2020. Smart Pet Feeder. International Online Conference on Engineering Response to COVID-19 (IOCER-COVID-19). 8-9 October 2020. West Bengal, India.

Vania, Karyono K., Nugroho I. H. T. 2016. Smart Dog Feeder Design Using Wireless Communication, MQTT and Android Client. International Conference on Computer, Control, Informatics and its Applications (IC3INA). 03-05 October 2016. Tangerang, Indonesia. 191.

Liu R. 2021. Automatic Pet Feeder based on Single Chip Microcomputer. Journal of Physics Conference Series. 2037(1):012104.

Airikala A.V., Prasetya H. C., Linggarjati J. 2020. Automatic Pet Feeder with Solar PV System. 4th International Conference on Eco Engineering Development. 10-11 November 2020, Banten, Indonesia.

Vrishanka P. N., Prabhakar P., Shet D., Rupali K. 2021. Automated Pet Feeder using IoT. 2021 IEEE International Conference on Mobile Networks and Wireless Communications (ICMNWC). 03-04 December 2021. Tumkur, Karnataka, India.

Kotcioğlu I. 2011. Clean and Sustainable Energy Policies in Turkey. Renewable and Sustainable Energy Reviews. 15(9): 5111-5119.

Yüksel A. N., Türkboyları E. Y. 2018. Use Of Solar Panels in Greenhouse Soil Disinfection. International Advanced Researches and Engineering Journal. 02(02): 195-199.

Kabalci Y., Kabalci E., Canbaz R., Calpbinici A. 2016. Design and Implementation of a Solar Plant and Irrigation System with Remote Monitoring and Remote Control Infrastructures. Solar Energy. 139 (2016): 506–517.

INVESTIGATION OF PADDY DRYING MACHINES AND ENERGY SOURCES USED IN DRYING

Aydın GULLU[1], *Aylin SATILMIS*[1]

[1] *Trakya University, Institute of Science, Mechatronic Engineering Department, Edirne, Turkey*
Corresponding author e-mail: aydingullu@trakya.edu.tr

ABSTRACT

The moisture content of the paddy plant after harvest is over 20%. This moisture content is not suitable for storage and processing. Therefore, the paddy plant needs to be dried. Approximately 600-1000 kg of paddy is harvested from an average of one acre of field. Drying must be fast for paddy harvested in large areas. Drying machines are used for this purpose. There are fixed or portable drying machines for paddy drying. These machines perform the moisture removal process by transferring hot air to the product with the support of a heat source. The paddy moisture removal process must be done at a certain time so that it does not damage the product. Therefore, the drying process of the machines is controlled. Various energy sources are used to provide hot air while drying with hot air. While it is possible to use energy sources such as electricity and fuel oil, they are less preferred due to the cost parameter. Drying can be done by burning coal or organic fuels efficiently. In this study, the structure and drying principles of paddy drying machines will be discussed. In addition, the energy sources used in these machines will be discussed.

Keywords: Paddy Drying, Energy Sources, Dryer Machine

INTRODUCTION

Rice, consumed by almost half of the world's population, is a basic need, source of nutrition and income. When the paddy is ripe, the ears are cut, and the grain consists of the caryopsis and the husk surrounding it without sticking to it and the inner husk. The product whose husk, that is, the husk, is not peeled is called paddy, the product that has only undergone the peeling process is called brown rice or cargo rice, and the final product that has undergone the peeling and polishing process is called rice. While 55-60% of rice is obtained during the processing of paddy, 15-20% is husk, 8-10% is bran, 7-8% is broken, 2% is damaged rice and 2% is obtained as grain product. (Taşlıgil & Şahin, 2011).
Rice (Oryza sativa L.) is a plant species from the Poaceae family and is an important export crop grown widely around the world. This plant is the only herbaceous warm climate plant cultivated in tropical and temperate water, they breathe and grow and develop using the dissolved oxygen in the water. Rice has the ability to grow in salty and alkaline soils, which is an important feature that distinguishes it from other plants and offers the potential to expand agriculture and increase productivity. (Öztürk & Akçay, 2010). The moisture content of the

products harvested from the field at 25-20% moisture should be reduced to 12-14% for storage and processing conditions. (Dizlek, 2014).

The agricultural sector includes a wide area with technological developments in food production worldwide, product flexibility and a perspective of expanding change. At this point, the emergence of continuous development requires the continuous renewal and development of the drying process in the rice sector. Because climate conditions, agricultural techniques and consumer expectations are constantly changing. Innovative solutions are constantly being sought in areas such as the development of new varieties, making drying processes more energy efficient, expanding automation and reducing environmental impacts. (Aydin & Güllü, 2007). Sustainability targets such as making waste management more effective in paddy drying systems should be set. It is important to minimize environmental impacts, protect natural resources and leave a healthier and safer world for future generations.
For a long time, it has been aimed to increase efficiency by using different techniques and resources in many parts of the world. Correct application of drying techniques protects the quality and consumability of grains while also minimizing product losses. In this study, the techniques used for drying paddy and the machines produced using these techniques will be discussed.

MATERIAL AND METHOD

Drying, dehumidification is the process of removing excess moisture from the product, a heat and mass transfer process. Heat transfer occurs from the product to the environment by convection, conduction or combinations. This transfer process from the product to the environment is affected by external factors such as the temperature of the drying environment, the humidity of the air, the type of product, the air flow rate and direction, and is also affected by internal factors such as the moisture content and temperature of the product. While external parameters affect the first stage of drying, internal parameters control the drying speed after reaching the critical moisture value. (Mengeş, 2005).
Common drying techniques in the agricultural industry and for food safety are listed below.

Drying Techniques

Air drying technique;

Cereals are drying in open areas using sunlight and natural ventilation. This method can be low-cost, but depends on weather conditions and the width of the drying area. The natural drying method in the sun can be effective in certain conditions, but it faces a number of technical limitations. In this method, the product exposed to direct sunlight creates uncertainty in the drying process. Lack of control in this process prolongs the drying time and creates uncertainty in the process of reaching the desired moisture level. In addition, the relative humidity level of the air may not be suitable for the product to be dried to reach the specified moisture level.(Suresh et al., 2023).

Mechanical drying;

Mechanical dryers blow hot air into the grains to remove moisture. This method is controlled and the air temperature and speed can be adjusted, thus providing fast and effective drying. There are many different machines in the industry for mechanical drying, with different capacities and different types of fuels. Zhang et al. (2022), analyzed the effects of different drying methods on the bursting rate, taste value and appearance quality to evaluate the quality of paddy rice in his studies. As a result of the analyses, a quality index system was used to comprehensively evaluate the quality of paddy rice. With the gray relational analysis method, the gray relational degree obtained when the moisture content was 24.4% during the drying process was determined as 0.996. These results show that the mechanical drying method gives the best result in terms of paddy rice quality.(Gummert, 2012).

Indirect drying technique;

Grains are dried not directly with hot air, but by the principle of heat conduction. Heat first heats a conductive surface and drying occurs by transferring the heat from this surface to the grains. Many studies have been conducted using different sources on indirect drying techniques. In a study conducted among solar drying techniques, El-Sebaey et al. (2023), observed that the indirect method resulted in higher quality products than other techniques. In Vitázek and Tirol (2008)'s study, they evaluated the performance of a closed grain dryer by measuring the temperature on the body surface with thermovision images and using the heat conduction principle.

Direct drying technique;

It is a technique in which grain products are dried by exposing them to direct heat or hot air flow (Babić et al., 2007). With this method, the products are placed directly into the dryer and heat or air is applied directly to the surface of the products, allowing the moisture to evaporate. Since heat or air comes into direct contact with the product surface, it provides rapid drying. One of the Italian Pedrotti Rice Dryer models used in this study uses special combustion chamber funnels to ensure that every calorie produced by the burners enters the product directly without heat loss with direct drying. The presence of heat distribution saves fuel. (Pedrotti, 2023).

Each drying method can work most effectively under certain conditions and requirements. Therefore, when choosing the right drying system, attention should be paid to the type of product to be used, its quantity and moisture content. In addition, the technologies used in rice drying depend on factors such as regional climate conditions, resource availability, access to technology and local production traditions. These differences significantly affect the sustainability, efficiency and quality of rice production. For example, Southeast Asia (SEA) contributes almost 20% to global rice production. (Nguyen-Van-Hung et al., 2019). In the Mekong River Delta of Vietnam, over 45% of rice is dried using mechanical dryers (Hung et al., 2013), in other regions (Filipinler, Myanmar) this rate drops to 5-10% or even below 5%. (Tado et al., 2015).

Drying techniques other than natural resources require a source for thermal energy. These sources can be electricity, natural gas, diesel, coal and other fossil fuels.

Drying Machines

There are several different types of machines used in the industry for drying paddy rice. These are flat bed, fluidized bed, rotary bed dryers. These machines are mostly found in factories. The most commonly used ones are vertical dryers that are operated mobile in the field or anywhere desired after harvest.

Flatbed dryers

Flatbed dryers (FBD) are used to dry paddy rice by spreading it on a flat bed. Some provide thermal energy from solar energy or natural air currents, while others use electricity, natural gas or paddy husk to provide energy. Figure 1 shows a picture of a flatbed dryer.

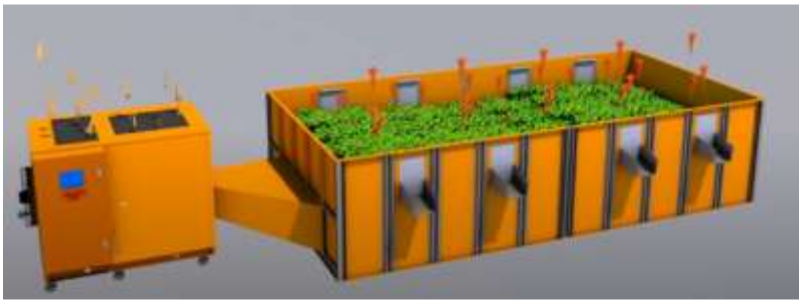


Figure 1. Flatbed Dryers

The rectangular bed of the flat bed founder is generally selected fine wire for air permeability (Syariffuddeen et al., 2020). After the drying air is heated, it is delivered to the grain pile by the fans and this process continues until the desired dem balance is achieved. FBD consists of three basic components, the oven to produce heat energy, the automation system for control, the fans to provide air flow and circulation, and the drying chamber. Flatbed dryer models vary depending on the air movements and direction. Models are available as single-direction (upward) air flow, double-direction (up and down) air flow (Hung et al., 2019).

Fluidized Bed Dryers

In fluidized bed dryers, the paddy is filtered in high-speed air currents. This filtration allows the paddy to dry quickly. These types of dryers are generally used on an industrial scale. An example of a fluidized bed dryer is given in Figure 2.

Figure 1. Fluidized Bed Dryers

In the fluidized bed system, air is pressurized with a fan inside the machine. The pressurized air passes through the perforated sheet and affects the product, affecting the gravitational force enough to suspend the grains. The aim here is to ensure that the product behaves like a fluid material (Kurtuluş, 2007). It has a wide range of uses, including processing, drying, cooling, agglomeration and granulation. (Ravi et al., 2023).

Rotary Bed Dryers

It is a type of dryer that tries to dry by moving it with a rotating drum. Heat and air flow distribute the grain evenly in the cylinder while drying it. It takes a certain amount of paddy, removes the paddy when the drying process is completed and makes a new load. Circulation is provided by continuous mixing during the process. The paddy pile is mixed homogeneously by shaking at a constant speed. This shaking process helps to balance the moisture accumulated on the surface of the paddy grains. (Firouzi et al., 2017). Energy is obtained by using fuels such as LPG, coal, husk, diesel. An example of a rotary bed dryer is given in Figure 3.

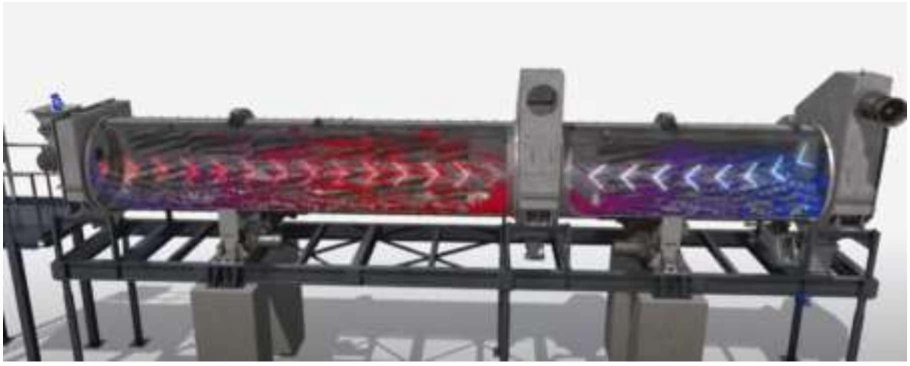


Figure 2. Rotary Bed Dryer

The leaves placed inside provide forward movement. In some dryers, a cooling process is performed as seen in the figure. This process is used to cool the grain after the drying process is completed and to quickly bring it back to ambient temperature. The air fan or cooling fan can also be called a cooling fan, which blows cold air to the material inside the rotating drum. This cold air prevents the product moisture from being reabsorbed. It also provides safe working conditions by preventing high temperatures that may occur inside the drum after the drying process.

Vertical Grain Dryer

Vertical grain dryers are other machines used to dry grains such as paddy, sunflower, corn. The vertical dryer is a mobile storage type system that sends the heat generated in the boiler to the heating chamber where the central helix is located via a low-pressure fan and presses it into the product at a certain pressure range from there, thus reducing the moisture of the product. Drying is done by changing the places of the product on the outside and the product that dries faster with the hot air spread from the inside to the outside, thus providing a homogeneous mixture. There is an 11 kW 1500 rpm feeding helix in the drying section of the vertical dryer. This helix conveys the paddy discharged into the hopper to the dryer. The main helix with a 22 kW 1000 rpm motor located in the center of the dryer carries the product from bottom to top in the dryer, thus providing drying. In addition, there is a 2.2 kW 1500 rpm motor dust fan outside the dryer, the fan increases efficiency by removing the garbage and grass in the paddy from the dryer. Since the garbage coming from the field with the product is wet like the paddy, it prolongs the drying period. Some producers clean the garbage in the paddy by sifting before drying. This is a necessary step because some factors such as moisture content changes and low drying temperatures during the process between harvest and storage put agricultural products under thermal and mechanical stress. Insufficient or no pre-treatment causes the drying period to be extended and causes excessive energy consumption. The dryer section of the general type of mobile vertical drying machine is given in Figure 4. The capacity starts from 5.5 tons and will dry 200 tons (daily) of paddy depending on the product type. The dryer used in this study has a 56m3 paddy chamber of approximately 31 tons. The basic points to be considered when choosing a dryer are to reach the maximum drying speed and to provide minimum energy consumption in obtaining the product with the desired properties. (Gungor & Ozbalta, 1997). The main factor is to use the heat in the dryer in the optimum way. The heat transfer varies according to the type of dryer, but it is a combination of transport, conduction and radiation. Many studies have been done and applied to determine the most suitable drying method. The drying method that increases energy efficiency and reduces environmental impacts without losing product quality is aimed (Kovacı et al., 2019).

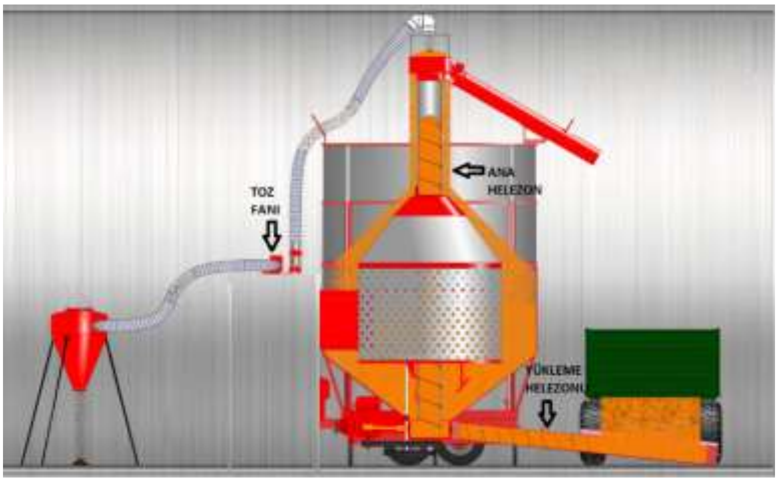


Figure 3. Vertical Grain Dryer

Drying machines generally do not operate with full energy efficiency due to inadequacies in theoretical calculations. Factors affecting energy efficiency can be listed as inadequate thermal insulation, drying methods that are not suitable for the product and environment, and poor quality energy sources (Jangam & Law, 2010). Increasing fuel costs and global warming have led to the most basic approach of recycling heat sources, thus energy efficiency can be greatly increased (Chua et al., 2010).

Examination of Fuels and Energy Resources

In this study, the thermal energy coming to the drying room is also provided by the energy released in the boiler. The energy of the boiler is approximately 700,000 kCal/hour. The product moisture entering the drying room with 1% moisture removal process per hour is realized within 6-12 hours depending on the outside air temperature and humidity rate. 45,000m3 of hot air at 65°C per hour is given into the dryer.
In paddy processing operating on an industrial scale, drying systems operating with coal, fuel oil, LPG fuels are used. The common point of these systems is to dry the paddy using high temperature and air flow. Fuel oil, which is widely used abroad as fuel, has led many drying facilities to turn to the coal-fired drying system, which is a more economically sustainable alternative, due to the energy consumption strategy based on import in our country, and the abnormal price increase arising from fluctuations in exchange rates.
Currently, fossil fuels are limited in terms of resources worldwide. These resources are rapidly depleting and cause environmental problems such as carbon dioxide emissions. Renewable energy sources and recyclable waste sources create a sustainable system.

Coal

The quality of coal depends on factors such as the amount of carbon in its organic matter and its moisture level. High carbon content directly affects the energy production potential of coal. Coal is divided into different classes according to the amount of carbon it contains. Generally, coal types are classified as "peat", "lignite", "hard coal" and "anthracite" according to their carbon content. Peat has a low carbon content (60% and below), while lignite has a medium carbon content (around 70%). Hard coal has a higher carbon content (80-90%), while anthracite is the type of coal with the highest carbon content (over 90%). High carbon content increases the combustion efficiency of coal, reduces the amount of coal used in energy production and thus minimizes environmental impacts. In addition, low moisture content increases the transportability and storability of coal. Therefore, quality standards and carbon content are decisive factors in the coal industry, because they directly affect the efficiency and environmental impacts in energy production processes. (Gunn et al., 2024).

Among coal types, anthracite, which ranks highest in terms of energy density, has a calorific value ranging from 7,300 to 8,000 kcal/kg and is used especially in industry and iron and steel industry. Second in line is hard coal, which has a calorific value ranging from 4,500 to 7,500 kcal/kg. A type of coal that is widely found around the world, hard coal has an important position among solid fuels. Although lignite constitutes approximately one third of the total world coal reserves, it is generally preferred in the power generation sector, namely thermal power plants, instead of heavy industry due to its low calorific value and high ash and moisture content. The calorific value of this brown coal, also known as lignite, is below 5,700 kcal/kg. Finally, peat is among the coal types that have completed their formation. Peat, which has an average calorific value below 2,600 kcal/kg, is generally found in humid regions. (Enerji & Bakanlığı).

Although our country is rich in coal deposits, a large part of its energy needs are provided from abroad, which causes risks in sustainable development and demand security. Although almost all of the existing conventional energy resources are available, only 30-31% of the total primary energy consumption is obtained from local resources. The low amount of energy obtained from own resources shows that a large part of the energy supply is based on imported energy resources. Turkey has approximately 2.1% of the world's coal reserves, and a significant part of these reserves consists of lignite. (MTA, 2023). Therefore, high energy fuels should be selected to provide the boiler energy and reach the moisture balance of the product within the specified time. In Turkey, this energy is reached with imported coal with high calorific value. Imported coal guarantees a lower calorific value of 7000-7800 Kcal//kg. This imported coal is burned approximately 40-50 kg to reduce the moisture content of paddy rice by 1% per hour. This value may vary depending on the conditions. Air temperature is also important among the factors affecting the total capacity and drying time. For example, since the air temperature drops at night, more energy needs to be produced, which means burning more coal.

Fuel oil

Fuel oil, the refining process involves the separation of crude oil into its different components through the thermal process of distillation. This process separates the components obtained from crude oil according to their boiling points. Fuel oil is produced during the distillation process, especially at temperatures between 250°C and 350°C. This temperature range is based on the evaporation and condensation properties of the various components in the crude oil. Fuel oil is a middle distillate product obtained at this stage of distillation and is generally used in various industrial applications such as industrial heating and power generation.

In grain drying plants, diesel fuel is usually stored and transported in large storage tanks. This gives plant operators the flexibility to provide fuel for long periods of operation. In terms of energy efficiency, for example, Pedrotti Grain Dryers, for a moisture range change of 28%-14%, the total kg of water to be evaporated per tonne is 163. The amount of water required to evaporate this water is 15l/tonne - 800Kcal/Kg H2O per tonne. In contrast, for a moisture range change of 24%-14%, the total kg of water to be evaporated per tonne is 116. The amount of water required to evaporate this water is 12l/tonne - 880Kcal/Kg H2O per tonne. The use of diesel fuel can cause emissions during the combustion process. These emissions can negatively affect air quality and have environmental impacts. It can often be more expensive than other types of fuel. However, the high energy density and efficiency of diesel fuel can reduce the total energy costs during the grain drying process (Pedrotti, 2023).
When evaluating the use of diesel fuel in vertical grain dryers in terms of energy values, it is very important to consider several factors. Studies have shown that diesel fuel consumption for drying grain varies depending on the moisture content of the grain pile and the efficiency of the dryer (Kuznetsov et al., 2023)
Direct combustion type burners are more common in diesel fuels. Specially designed combustion chamber funnels are used to ensure that every calorie is delivered to the crop without heat loss. Figure 5 shows the working method of a direct combustion type burner. Although burners provide advantages in terms of efficiency, their prevalence is not equal among countries due to the high cost of fuel. The high cost of diesel fuel in Turkey directs consumers to other cheaper alternatives.

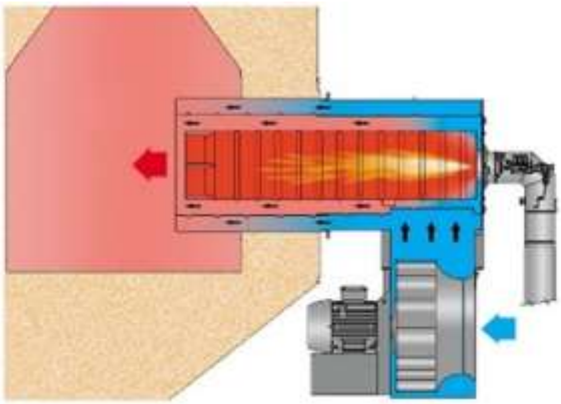


Figure 4. Direct Combustion Type Burner (Pedrotti, 2023)

Rice Husk

Husk is an important by-product of the rice industry and can be used as fuel in various industrial processes. When used as fuel, the technical properties of husk should be taken into account. Factors such as the combustion behavior of husk, the composition of gases formed during combustion and combustion efficiency are important.
Paddy husk can be considered as an alternative fuel due to its high silicon content. Although these husk are considered as waste after paddy production, they have various uses and new areas of use are being investigated. Paddy husk has a calorific value of 3000kCal/kg. The

chemical composition of burnt husk is approximately 70% SiO_2 (Martinez et al., 2006). In addition, if we look at the energy obtained by burning the husk, the energy obtained per kilogram varies between 2.6MJ and 7.5MJ (Steven et al., 2022). The flue gas formed as a result of husk combustion constitutes approximately 14% of CO_2, which is considered a greenhouse gas. The ash content contains a large amount of silicon. The combustion properties of husk determine its usability as a fuel. These properties include factors such as the moisture content of the husk, its calorific value, its ash content and the emission profile of the gases formed during combustion. In addition, the physical properties of husk are also important, because the fuel must burn homogeneously during the combustion process and the combustion chamber must be used effectively. The advantages of using husk as a fuel include being a renewable resource, low cost and contributing to waste management. However, the combustion properties and technical requirements of husk should be taken into consideration. Therefore, suitable combustion systems and designs should be developed for the use of husk as a fuel. In this way, the potential of husk, which is a rice husk fuel, can be evaluated more effectively. In addition, it is important to use this product to reduce zero waste and environmental pollution. (Akyol et al., 2016).

Other Fuels

Grain dryers can utilize a variety of fuel sources to operate efficiently. Common fuels include LPG, coal, wood, coconut shells, and solar energy. LPG and coal are traditional fuel sources used in industrial continuous flow grain dryers (Dębowski et al., 2021) . Wood, especially in the form of firewood or sawdust, is a common fuel source for grain dryers in countries such as Brazil. (Lima et al., 2017), (Quequeto et al., 2022), (Resende et al., 2022). Coconut shells have also been investigated as an alternative heat energy source for grain dryers (Dermawan et al., 2022). In addition to traditional fuel sources, renewable energy sources such as solar energy are also being evaluated to power grain dryers. In addition, connecting energy sources such as solar energy are also being evaluated to power the dryers (Jiang et al., 2010). In addition, studies are being carried out to reduce power cuts and energy savings through the use of electrophysical (Sjechlad et al., 2019). These disruptions are focusing on the development of new improvements to optimize energy consumption in dryers and minimize concentration effects (Sjechlad et al., 2019).

CONCLUSIONS

Rice harvested from the field with more than 20% moisture needs to be dried. This process needs to be done quickly, with minimal damage to the product. Different methods have been developed according to the incoming moisture structure and the place of use. Air drying, mechanical drying, indirect drying and direct drying techniques have been developed to remove water from the product. Drying process has been carried out by developing machines that work with these techniques. Flat bed, fluidized bed, rotary bed and vertical grain dryers have been developed considering the conditions such as the incoming grain type and the place of operation. Drying is provided by giving hot fluid to the product in these machines. Rice is in high humidity after harvest. It is important to remove this moisture quickly. Vertical mobile grain dryers that farmers can use have been developed for this purpose. An energy source is required for drying in these mobile machines. Fuel types used in grain dryers vary from traditional sources such as LPG and coal to alternative sources such as wood, coconut shells, pellets and renewable sources such as solar energy. When drying paddy rice harvested from the field with moisture above 20%, fuel selection can affect the efficiency, cost-effectiveness and environmental sustainability of the grain drying process.

REFERENCES

Akyol, E., İsmet, Y., Koçer, A., Güngör, A., & Hacıhafızoğlu, O. (2016). Sabit yataklı gazlaştırıcı açısından Trakya Bölgesi biyokütle potansiyeli. *Küresel Mühendislik Çalışmaları Dergisi*, *3*(2), 155-162.

Aydin, E., & Güllü, A. (2007). A Survey: Sustainable Agricultural Policies In İpsala. *Education*, *2011*.

Babić, M., Babić, L., & Minute, G. (2007). Tehničke specifičnosti novije generacije sušara za zrno. *Časopis za procesnu tehniku i energetiku u poljoprivredi/PTEP*, *11*(4), 203-207.

Chua, K. J., Chou, S. K., & Yang, W. M. (2010). Advances in heat pump systems: A review. *Applied Energy*, *87*(12), 3611-3624. https://doi.org/https://doi.org/10.1016/j.apenergy.2010.06.014

Dębowski, M., Bukowski, P., Kobel, P., Bieniek, J., Romański, L., & Knutel, B. (2021). Comparison of Energy Consumption of Cereal Grain Dryer Powered by LPG and Hard Coal in Polish Conditions. *Energies*, *14*(14), 4340. https://doi.org/10.3390/en14144340

Dermawan, D., Nur, R., Susanto, T. A., & Amrullah, A. (2022). Designing and Manufacturing of Grain Dryer Machine Using Coconut Shell Fuel as an Alternative Heat Energy. *Intek Jurnal Penelitian*, *8*(2), 169. https://doi.org/10.31963/intek.v8i2.3351

Dizlek, H. (2014). Depolama sırasında tahıllarda meydana gelen fiziksel ve kimyasal değişiklikler. *Gıda ve Yem Bilimi Teknolojisi Dergisi*(12).

El-Sebaey, M. S., Mousavi, S. M. T., Shams El-Din, S., & Essa, F. A. (2023). An experimental case study on development the design and the performance of indirect solar dryer type for drying bananas. *Solar Energy*, *255*, 50-59. https://doi.org/https://doi.org/10.1016/j.solener.2023.03.023

Enerji, T. C., & Bakanlığı, T. K. Kömür. *ENERJI. gov. tr Available: https://www. enerji. gov. tr/File*.

Firouzi, S., Alizadeh, M. R., & Haghtalab, D. (2017). Energy consumption and rice milling quality upon drying paddy with a newly-designed horizontal rotary dryer. *Energy*, *119*, 629-636.

Gummert, M. (2012). Improved postharvest technologies and management for reducing postharvest losses in rice. II Asia Pacific Symposium on Postharvest Research Education and Extension: APS2012 1011,

Gungor, A., & Ozbalta, N. (1997). Endüstriyel Kurutma Sistemleri (In Turkish). III. *Ulusal Tesisat Mühendisliği Kongresi ve Sergisi, Bildiriler Kitabı, II. Cild*, 737-747.

Gunn, P., Ashley, G., & Mackenzie, H. (2024). Coal basics. Coal Marketing International Ltd. In.

Hung, N., Hung, N., Truong, T., & Hien, P. (2013). Drivers of Postharvest Development and Milestones of ADB-IRRI Rice Postharvest Project in Vietnam. In.

Hung, N. V., Martinez, R., Tuan, T. V., & Gummert, M. (2019). Development and verification of a simulation model for paddy drying with different flatbed dryers. *Plant Production Science*, *22*(1), 119-130.

Jangam, S. V., & Law, A. S. M. C. L. (2010). Drying of foods, vegetables and fruits. In.

Jiang, T., Li, G., Wang, H. T., Zhang, K. C., & Zhang, Y. B. (2010). Composite Agglomeration Process (CAP) for Preparing Blast Furnace Burden. *Ironmaking & Steelmaking*, *37*(1), 1-7. https://doi.org/10.1179/174328109x462995

Kovacı, T., Dikmen, E., & Şencan Şahin, P. D. A. (2019). Kurutma sistemleri, enerji tüketimleri ve ürün kalitesine etkileri ve örnek sistem tasarımı.

Kurtuluş, O. (2007). Akışkan yatakta kurutma prosesinin incelenmesi.

Kuznetsov, N., Vershinin, V., Nikiforov, V., Симонов, Г. А., & Filippova, O. B. (2023). Improving the Efficiency of Drying Devices by Using the Heat of the Spent Drying Agent. *Science in the Central Russia*. https://doi.org/10.35887/2305-2538-2023-1-34-42

Lima, R. F. d., Dionello, R. G., Peralba, M. d. C. R., Barrionuevo, S., Radünz, L. L., & Júnior, F. W. R. (2017). PAHs in Corn Grains Submitted to Drying With Firewood. *Food Chemistry*, *215*, 165-170. https://doi.org/10.1016/j.foodchem.2016.07.164

Martinez, V., Valencia, M., Cruz, J., Mejia, J., & Chejne, F. (2006). Production of β-SiC by pyrolysis of rice husk in gas furnaces. *Ceramics international*, *32*(8), 891-897.

Mengeş, G. (2005). Patatesin farklı kurutma şartlarındaki kurutma karakteristiklerinin belirlenmesi. *Selçuk Üniversitesi Fen Bilimleri Enstitüsü Yayınlanmamış Yüksek Lisans Tezi, Konya*.

MTA. (2023). *Kömür Arama Araştırmaları*. https://www.mta.gov.tr/v3.0/arastirmalar/komur-arama-arastirmalari

Nguyen-Van-Hung, Tran-Van-Tuan, Meas, P., Tado, C. J. M., Kyaw, M. A., & Gummert, M. (2019). Best practices for paddy drying: case studies in Vietnam, Cambodia, Philippines, and Myanmar. *Plant Production Science*, *22*(1), 107-118. https://doi.org/10.1080/1343943X.2018.1543547

Öztürk, D., & Akçay, Y. (2010). Güney Marmara Bölgesinde çeltik üretiminin genel bir değerlendirmesi. *Journal of Agricultural Faculty of Gaziosmanpaşa University (JAFAG)*, *2010*(2), 61-71.

Pedrotti. (2023). *Energy Saving*. https://pedrottisrl.it/features/energy-saving/

Quequeto, W. D., Resende, O., Tfouni, S. A. V., Gomes, F. M. L., Borges, A. X., Almeida, A. B. d., Cabral, J. C. O., Costa, L. M., & Oliveira, L. P. d. (2022). Corn Grains Drying Using Direct-Fired Furnace With Wood Chips: Performance, Quality and Polycyclic Aromatic Hydrocarbons. *Food Science and Technology*, *42*. https://doi.org/10.1590/fst.27122

Ravi, N., Naik, S. S., & Dharavath, I. (2023). Study on Sorghum Grains Drying in a Fluidized Bed Dryer.

Resende, O., Costa, E. R. d. O., Quequeto, W. D., Costa, L. M., Oliveira, D. E. C. d., Tfouni, S. A. V., Gomes, F. M. L., Quirino, J. R., & Lima, R. R. (2022). Quality of Corn Grains

Subjected to Drying Using Direct-Fired Furnace Fed With Eucalyptus Chips and Firewood. *Food Science and Technology*, *42*. https://doi.org/10.1590/fst.55820

Sjechlad, D. Z., Jamari, J., & Widyanto, S. A. (2019). Auto-Stirring Grains Bed Dryer as an Innovative Efficiency Solution. *E3s Web of Conferences*, *125*, 13001. https://doi.org/10.1051/e3sconf/201912513001

Steven, S., Hernowo, P., Restiawaty, E., Irawan, A., Rasrendra, C. B., Riza, A., & Bindar, Y. (2022). Thermodynamics simulation performance of rice husk combustion with a realistic decomposition approach on the devolatilization stage. *Waste and Biomass Valorization*, 1-13.

Suresh, B. V., Shireesha, Y., Kishore, T. S., Dwivedi, G., Haghighi, A. T., & Patro, E. R. (2023). Natural energy materials and storage systems for solar dryers: State of the art. *Solar Energy Materials and Solar Cells*, *255*, 112276. https://doi.org/https://doi.org/10.1016/j.solmat.2023.112276

Syariffuddeen, A., Yahya, S., Ruwaida, A., Zainun, M., Shahrir, A., Azman, H., Shafie, A., Zaimi, Z., Hafiz, M., & Amir Redzuan, S. (2020). Evaluation on drying temperature of grain corn and its quality using flat-bed dryer. *ASM Sc. J.*

Tado, C., Ona, D. P., Abon, J. E. O., Gagelonia, E., Nguyen, T. N., & Le, Q. (2015). Development and promotion of the reversible airflow flatbed dryer in the Philippines. *Annals of Tropical Research*, *37*(1), 97-109.

Taşlıgil, N., & Şahin, G. (2011). Türkiye’de çeltik (Oryza sativa L.) yetiştiriciliği ve coğrafi dağilimi. *Adıyaman Üniversitesi Sosyal Bilimler Enstitüsü Dergisi*(6), 182-203.

Vitázek, I., & Tirol, J. (2008). Relation between surface temperature and dryer operation. *Research in Agricultural Engineering*, *54*(4), 176-182.

Zhang, N., Wu, W., Li, S., Wang, Y., Ma, Y., Meng, X., & Zhang, Y. (2022). Comprehensive Evaluation of Paddy Quality by Different Drying Methods, Based on Gray Relational Analysis. *Agriculture*, *12*(11), 1857.

FREQUENCY OF USE OF MACHINE LEARNING METHODS IN VARIOUS SCIENTIFIC FIELDS

Tuna TOPAÇ[1], Özlem UÇAR[2], Erdem UÇAR[1]

[1] *Trakya University, Faculty of Applied Sciences, Management Information Systems Department, Edirne, Turkey*
[2] *Trakya University, Engineering Faculty, Department of Computer Engineering, Edirne, Turkey*

Corresponding author e-mail: ozlemu@trakya.edu.tr

ABSTRACT

Researchers who use artificial intelligence techniques in their studies have carried out academic studies by specifically incorporating machine learning, which is a sub-branch of this field, into their studies. In these studies, stereotyped algorithms in machine learning were generally used. The algorithms, models and methods used have tasks such as regression, classification, clustering and dimensionality reduction. Related studies vary depending on the area used and the needs. In this study, data about the algorithms, models, methods and usage amounts used in the publications published between 2019 and 2023, where the concept of machine learning is available in the DergiPark database, were processed and tables regarding the usage amounts were made, and a detailed comparison was made about how frequently the algorithms, models and methods were used in which areas. The method used in this study is to search for the specified keyword from the DergiPark database, download articles related to machine learning, scan them one by one, and record the algorithm numbers. Data were collected in three main areas: science, social and health. Science and social sciences are also divided into subfields. 692 articles were scanned: 273 articles in science, 259 articles in social sciences, and 160 articles in health sciences. 39 of these studies belong to the agricultural field. The algorithms used in studies in the field of agriculture are mainly of the supervised learning type, and deep learning has an important place in this field. The aim of the study is to shed light on which machine learning method can be used more efficiently for researchers in this field.

Keywords: Artificial Intelligence, Machine Learning, Agriculture, Data, Statistic

INTRODUCTION

Machine learning is not only an important decision tool for agriculture but also siding decisions on crop classification, performance on clustering crops, what crops to grow and what to do while supporting farms and villages around the country also detecting disease in plants, exploring smart agriculture, optimizing water management system and energy [1][2][3][4]. Several machine learning algorithms have been applied to support this field of prediction research. Machine learning methods, which are based on statistics, eliminate the limitations of statistical methods in analysis and provide researchers from all fields with the opportunity to perform more comprehensive and flexible analyses in today's abundance of data. But statistic ought to promote data of what artificial intelligence methods used in academy especially in agriculture. Today, the development of technology and the rapid increase in data sizes have caused different concepts to enter artificial intelligence field. These concepts vary according to their use cases and using frequencies. The penetration of information and communication technologies into almost every area of life and rapid technological developments trigger an increase in data sizes and types. For this reason, processing data is becoming more and more important every day to obtain meaningful and useful information from among data piles. In data analysis, statistics, which is the science of interpreting data and basing it on evidence, can limit researchers and be inadequate in the face of large-scale data sets. Although statistical methods are used in many fields, the concept of machine learning, which is based on statistical methods in data analysis, has emerged and enables process and analysis of different types of large-scale data, both structural and non-structural. Although machine learning and statistics serve similar goals, there are differences between them. Statistics, as a branch of science that examines the relationships between variables, analyzes the data set with various calculations and summarizes the findings, and generalizes the results obtained from the sample for the data mass. In this work, statistic helped us for making sense of where machine learning methods used. A literature review was conducted to create this study. A total of 692 sources written in Turkish and English including conference proceedings, articles published in DergiPark between 2019 and 2023, were examined. In the searches made on the DergiPark search engine, the keywords "Makine Öğrenmesi" were used and the results were scanned and sources that were found suitable for the study were examined. Figure 1 shows framework of survey.

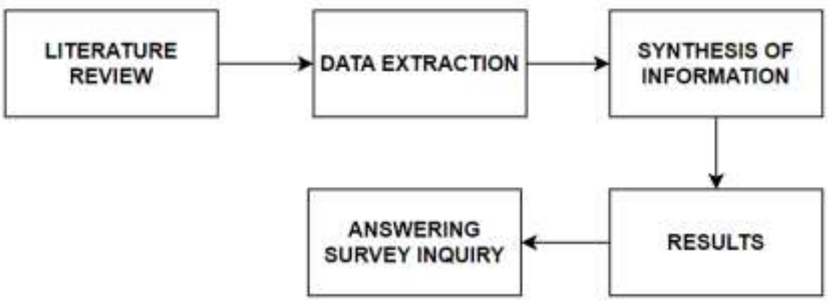


Figure 1. Framework of survey’s mechanism

MACHINE LEARNING

Machine learning, a sub-branch of artificial intelligence, uses statistics and computer processing power to detect patterns in complex data and make rational decisions [5]. Machine learning algorithms use a variety of statistical, probabilistic, and optimization methods to learn from past experiences and detect useful patterns in large, unstructured, and complex data sets.

In summary, machine learning is the modeling of systems that make inferences and predictions from data using statistical and mathematical operations, using computers [6]. Machine learning algorithms are divided into 3 groups according to the way they learn from data: Supervised, unsupervised and reinforcement learning. Supervised learning is a learning method that is performed by knowing what the results of the data inputs used in the algorithms will be when creating machine learning models. In other words, in this learning method, the input (Independent variables) and output (Dependent variable) are known. The outputs are the labels of the data. This type of learning is used in classification and regression algorithms. Unsupervised learning is a learning method in which the labels, that is, the classes, of the data are not known and models are created according to their similar features. In this learning method, there is only input. The algorithm creates the model without labels. Clustering and dimensionality reduction are examples of unsupervised learning. Reinforcement learning is the creation of a model by exposing and training an actor to the concepts of reward and punishment as a result of his actions. Optimization algorithms can be given as examples of this learning method. Deep learning is a form of learning that aims to make sense of the whole data with the help of layers of artificial neural network architecture created by taking the human brain as an example, taking into account the relationships of small pieces of data with each other.

Artificial Neural Networks

Artificial neural networks are a machine learning algorithm that mimics the idea of the working principle of the human brain. In this architecture, units called neurons process inputs according to the weights in the neurons and provide an output. Artificial neural networks are an excellent fault-tolerant and very fast architecture when processed in parallel. It extracts significant information from small relationships in the data [7].

Boosting Algorithms

Boosting algorithms are used in classification and regression architectures to improve the performance of the algorithm. This is done by updating the weights in each iteration. The process continues until the quality of the algorithm is optimized [8].

Convolutional Neural Networks

Convolutional neural networks are a special type of artificial neural networks. This architecture, which is generally used in image processing, is also used in natural language processing such as text processing. The most important feature of this architecture is that it allows the information to be used and summarized in the most efficient way with the help of the image grid structure [9].

Decision Trees

Decision trees are algorithms used in machine learning for classification tasks. They generally work with the divide and conquer logic. They can extract and reveal elements in large databases [10].

K-Means

The K-means algorithm is an unsupervised machine learning algorithm that clusters and classes unlabeled data based on Euclidean distance [11].

K Nearest Neighbor

K Nearest Neighbor is a supervised machine learning and data mining algorithm used in classification problems with its easy implementation and remarkable performance. The algorithm is the process of determining the closest input that is like the formed classes [12].

Linear Regression

Linear regression is a regression machine learning algorithm that consists of one dependent and one independent variable that is created as needed in models with ongoing continuous data [13].

Logistic Regression

Logistic regression is a supervised learning algorithm used in classification. The aim of the algorithm is to optimize a 2-class space and give appropriate outputs [14].

Naïve Bayes

Although it does not show much accuracy in real-world applications, it is a machine learning algorithm used in naive bayes classification, whose working logic is that the probability of an event occurring depends on the probability of any other event occurring [15].

Principal Component Analysis

Principal component analysis, namely, is an information extraction algorithm that works by changing the positions of the axes in the plane according to the situation in order to reveal important information in the data set on a certain plane [16].

Random Forest

Random forest is a powerful machine learning algorithm used in classification problems where the judgment of multiple decision trees is used in cases where there are unobservable variables in the environment [17].

Recurrent Neural Network

Recurrent neural network, as its name suggests, is a deep learning architecture used in situations where repetitive data is the issue. Repetitive data refers to structures such as time series and text data [18].

Sequential Minimal Optimization

The Sequential Minimal Optimization algorithm is a machine learning algorithm used specifically to improve the performance of support vector machines [19].

Support Vector Machines

Support vector machines are a popular algorithm that is often mentioned among classification algorithms, especially in cases where binary classes are present. The flexibility in the algorithm has made it possible to adapt to many situations. In this way, it plays an important role not only in binary classification but also in other classifications [20].

SOME STUDIES CONDUCTED IN DERGİPARK

Agricultural product pattern detection was performed in the Çivril-Baklan Plain within the borders of Çivril, Baklan and Çal districts of Denizli province, using 2020 Sentinel-2 satellite images and the python-based open source Eo-Learn library within Light Gradient-Boosting algorithm [21].

Tree Seed Algorithm is applied to clustering problems and the obtained results are compared with the traditional methods widely used in clustering process. It has been shown that Tree Seed Algorithm provides better search locally and globally thanks to its exploration and processing characteristics and can reach better results than the results achieved by traditional clustering methods [22].

The impacts of some environmental element on the weaning weights of Awassi lambs were examined as prediction was made by the regression decision tree method, that is one of the machine learning algorithms [23].

River flow data from consecutive flow monitor stations in the Eastern Mediterranean Basin and Seyhan Basin located on the same river routeing were used. In the literature, there are various previous step numbers in the input parameters as time series, and it was determined that the inputs of 6 months and earlier did not create much difference in the results compared to the previous model evaluations [24].

ANFIS method, one of the machine learning methods, was used to estimate the amount of sunflower production. To increase the finding out ability of the ANFIS model, the membership function numbers of the input variables were determined with the K-means algorithm [25].

Genotype yield estimates were made using regression-based algorithms within machine learning methods, using yield information of the genotypes used in the 2018 barley breeding program, collected from 12 different locations in 24 genotypes with 4 replications [26].

A deep feature extraction and machine learning based method was developed to detect diseases on plant leaves. To test the proposed method, 726 images of walnut leaves and a two-class dataset were collected. Feature extraction was performed on the images with DarkNet53 and ResNet101 models. The obtained features were combined, and the most weighted features were selected with the ReliefF algorithm. The selected features were classified with the SVM algorithm [27].

Data from 54 calves, 70% of which is the training set and 30% of which is the test set, was used with the artificial neural network. Illative of the analysis, the highest correct classification rate of diseased and healthy calves was calculated using the artificial neural network method [28].

Performance of 3 distinct algorithms for machine learning was measured from raisin images and morphological features extracted from these resulting images [29].

Histogram Based Gradient Augmentation and Support Vector Machine were applied in regression analyses for yield estimation [30].

Models for predicting soil moisture, which plays an important role in agricultural practice, were introduced. The accuracy of these models was compared using the coefficient of determination and the mean square error. The data acquired from the system enhanced for 55 days is used to train and test the models. The algorithms of multiple linear regression, polynomial regression, support vector regression, decision tree regression and random forest regression were used for estimation [31].

A convolutional network model was built from ground up and it was trained to conduct the required detection of plant diseases in the given set of classes. It was showed that the model has better classification capability than previous models [32].

The aim was to model the drying behavior of an organomineral fertilizer, which is one of the most important problems in the agricultural industry. Not only regression, but also the performances of multilayer perceptron and long short-term memory models were investigated. As a regression method, exponential function was used. In addition, Gaussian regression based on artificial neural network with covariance kernel function was also investigated [33].

The use of multitemporal Polarimetric Synthetic Aperture Radar images in the classification of agricultural products was investigated and the classification performances of machine learning algorithms used for this purpose were compared. Although the highest classification accuracy was obtained with the Light Gradient-Boosting Machine algorithm, it was observed that the difference between it and the random forest algorithm was not statistically significant [34].

In order to determine the land cover change in Kocaeli province due to urbanization and agricultural activities within the framework of dynamic change determination according to LandCover 2.0 standards, multi-temporal Sentinel 2 satellite images were used to comparatively evaluate the performance of four different data reduction - classification method combinations, namely, Location Index-Random Forest, Principal Component Analysis-

Random Forest, Location Index-Regression Tree and Principal Component Analysis-Regression Tree [35].
An approach based on the Faster Local-Convolutional Neural Networks model of deep learning has been proposed for the identification of cattle based on their facial images. As a result of experimental studies conducted on the test dataset, a classification performance of 98.44% accuracy has been achieved [36].
Classification was performed using a publicly available dataset containing 5 flower classes consisting of 4317 flower images, using deep learning methods and feature selection methods together [37].
The Dynamic Water Budget model modeled the basin rainfall-runoff relationship, which the conceptual rainfall-runoff model simulated through various parameters, more reliably in both calibration and validation periods compared to the Support Vector Regression based machine learning model [38].
The performances of the new generation ensemble learning algorithms XGBoost and LightGBM in the classification of agricultural products were compared. This performance comparison was made based on both model accuracy and processing time. Our experimental results concluded that the LightGBM method is superior to the XGBoost method in the classification of agricultural products [39].
Models were developed to estimate the breast diameter using the stump diameter value of stone pine, an important forest tree species, to be used within the administrative borders of the Izmir Regional Directorate of Forestry. The equations defining the relationship were obtained using the regression analysis method. The data set consists of measurement values obtained from 266 sample trees [40].
An image processing system was designed to discriminate among two different raisin varieties cultivated in Turkey. The collected images were applied to different pre-processing steps and 7 feature extraction morphological operations were conducted using image processing techniques. Using logistic regression, multilayer perceptron and machine learning with support vectors, models were constructed, and performance measurements were made [41].
The classification of apricot kernels as bitter and sweet was done using image processing and machine learning algorithms. The results obtained using seven different machine learning algorithms were compared [42].

ALGORITHMS ACCORDING TO USE CASE

The algorithms used in the studies vary according to their tasks and the type of data set. Recently popular deep learning architecture and support vector machines, which are frequently used in classification problems, have come to the forefront in terms of frequency of use. Classification is a type of supervised learning used especially when product and soil diversity come to the fore. They were followed by regression algorithms. Regression architecture has come to the fore in problems where various hybrid algorithms are used. Regression was used in problems where water regimes and production tracking were made according to years. Collecting and classifying agricultural data is not an easy task. For this reason, unsupervised learning methods used in machine learning cope with this task. In the studies in this article, clustering algorithms were used, although in small numbers. Figure 2 shows usage tasks according to the types of mentioned algorithms. Figure 3 shows distribution of machine learning methods used in studies conducted in the field of agriculture between 2019 and 2023 in DergiPark according to their count of use.

Figure 2. Usage tasks according to the types of mentioned algorithms

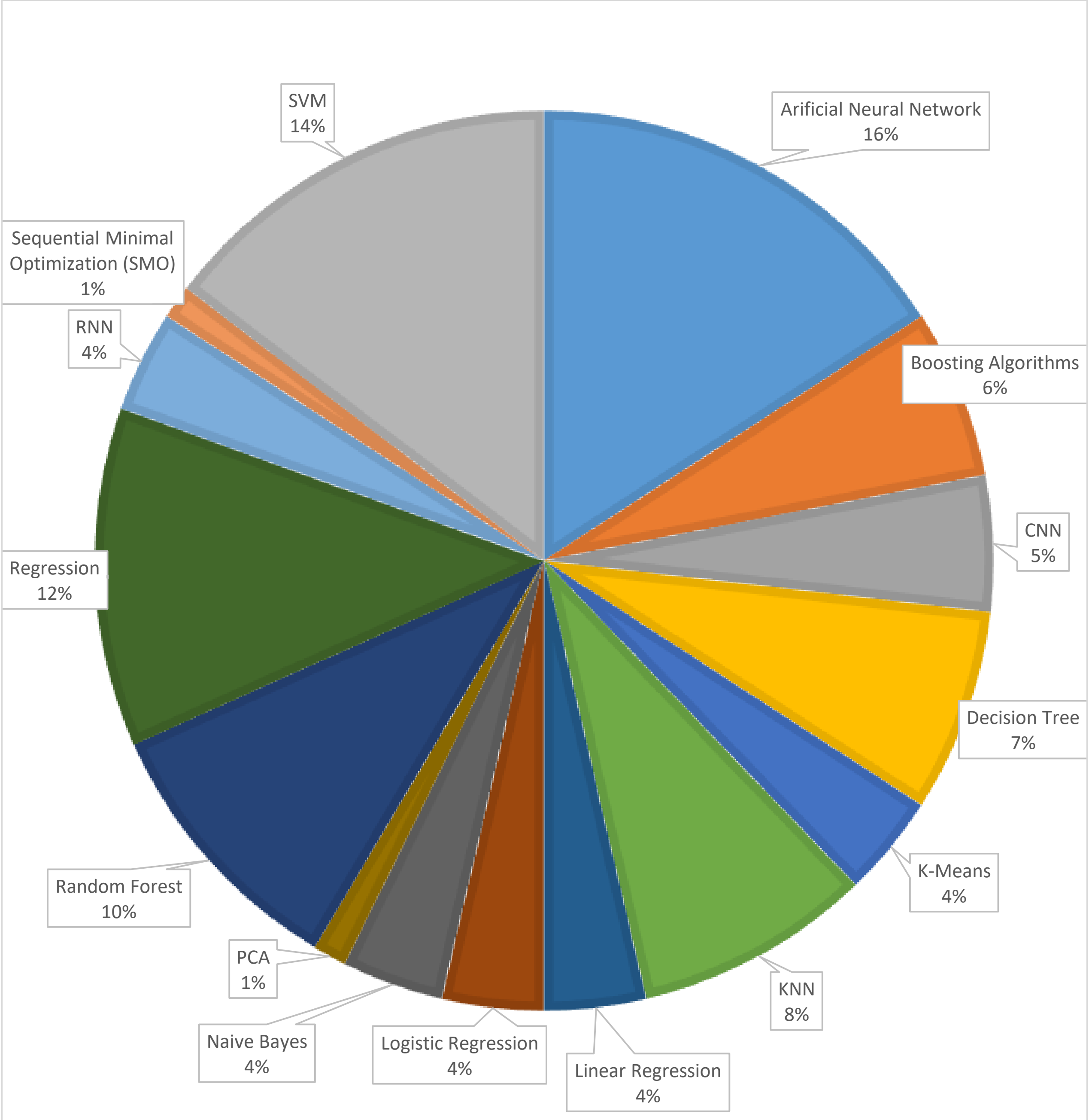


Figure 3. Distribution of Machine Learning Methods Used in Studies Conducted in the Field of Agriculture Between 2019 and 2023 in DergiPark According to Their Count of Use [21], [22], [23], [24], [25], [26], [27], [28], [29], [30], [31], [32], [33], [34], [35], [36], [37], [38], [40], [41], [42], [43], [44], [45], [46], [47], [48], [49], [50], [51], [52], [53], [54], [55], [56], [57], [58], [59]

CONCLUSION

In the study, it was seen that 95% of the methods used in the field of agriculture fall into the supervised learning class. Only 5% of them worked in this field using unlabeled data. The reason why supervised learning methods are preferred depends on the type of problem selected, the performance and consistency criteria being significantly higher in these machine learning methods, and the type of data used. In future, with arrival of 5G and IoT technologies to Turkey machine learning should be utilized much more effectively in agriculture. It will impact the economy of the country.

REFERENCES

[1] M. Shoaib *et al.*, "An advanced deep learning models-based plant disease detection: A review of recent research," 2023, *Frontiers Media SA*. doi: 10.3389/fpls.2023.1158933.

[2] T. van Klompenburg, A. Kassahun, and C. Catal, "Crop yield prediction using machine learning: A systematic literature review," Oct. 01, 2020, *Elsevier B.V.* doi: 10.1016/j.compag.2020.105709.

[3] D. Bini, D. Pamela, and S. Prince, "Machine Vision and Machine Learning for Intelligent Agrobots: A review," in *ICDCS 2020 - 2020 5th International Conference on Devices, Circuits and Systems*, Institute of Electrical and Electronics Engineers Inc., Mar. 2020, pp. 12–16. doi: 10.1109/ICDCS48716.2020.243538.

[4] D. Maria Manuel Vianny, A. John, S. Kumar Mohan, A. Sarlan, Adimoolam, and A. Ahmadian, "Water optimization technique for precision irrigation system using IoT and machine learning," *Sustainable Energy Technologies and Assessments*, vol. 52, Aug. 2022, doi: 10.1016/j.seta.2022.102307.

[5] C. ÇOŞKUN and E. YÜKSEK, "Hepatit hastalığının tespitinde bulanık mantık ve makine öğrenmesi yöntemlerinin karşılaştırılması," *DÜMF Mühendislik Dergisi*, Nov. 2023, doi: 10.24012/dumf.1319102.

[6] O. YILDIRIM, F. B. GUNAY, and M. YAĞANOĞLU, "Makine Öğrenmesi Yöntemleriyle Orman Yangını Tahmini," *Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, vol. 13, no. 3, pp. 1468–1481, Sep. 2023, doi: 10.21597/jist.1249908.

[7] J. Zou, Y. Han, and S. S. So, "Overview of Artificial Neural Networks," *Methods in Molecular Biology*, vol. 458, pp. 14–22, 2008, doi: 10.1007/978-1-60327-101-1_2.

[8] M. V. Joshi, V. Kumar, and R. C. Agarwal, "Evaluating boosting algorithms to classify rare classes: Comparison and improvements," *Proceedings - IEEE International Conference on Data Mining, ICDM*, pp. 257–264, 2001, doi: 10.1109/ICDM.2001.989527.

[9] S. Sakib, N. Ahmed, A. J. Kabir, and H. Ahmed, "An Overview of Convolutional Neural Network: Its Architecture and Applications," Feb. 2019, doi: 10.20944/PREPRINTS201811.0546.V4.

[10] A. J. Myles, R. N. Feudale, Y. Liu, N. A. Woody, and S. D. Brown, "An introduction to decision tree modeling," *J Chemom*, vol. 18, no. 6, pp. 275–285, 2004, doi: 10.1002/CEM.873.

[11] B. Chong, "K-means clustering algorithm: a brief review," *Academic Journal of Computing & Information Science*, vol. 4, pp. 37–40, 2021, doi: 10.25236/AJCIS.2021.040506.

[12] S. Zhang, X. Li, M. Zong, X. Zhu, and D. Cheng, “Learning k for kNN Classification,” *ACM Transactions on Intelligent Systems and Technology (TIST)*, vol. 8, no. 3, Jan. 2017, doi: 10.1145/2990508.

[13] X. Su, X. Yan, and C. L. Tsai, “Linear regression,” *Wiley Interdiscip Rev Comput Stat*, vol. 4, no. 3, pp. 275–294, May 2012, doi: 10.1002/WICS.1198.

[14] M. P. LaValley, “Logistic regression,” *Circulation*, vol. 117, no. 18, pp. 2395–2399, May 2008, doi: 10.1161/CIRCULATIONAHA.106.682658/ASSET/472996AA-9EBA-4322-A948-192E511AC922/ASSETS/GRAPHIC/19FF1.JPEG.

[15] H. Zhang, “The Optimality of Naive Bayes,” 2004, Accessed: Aug. 31, 2024. [Online]. Available: www.aaai.org

[16] H. Abdi and L. J. Williams, “Principal component analysis,” *Wiley Interdiscip Rev Comput Stat*, vol. 2, no. 4, pp. 433–459, Jul. 2010, doi: 10.1002/WICS.101.

[17] G. Biau and E. Scornet, “A random forest guided tour,” *Test*, vol. 25, no. 2, pp. 197–227, Jun. 2016, doi: 10.1007/S11749-016-0481-7/FIGURES/4.

[18] M. S. Alam, F. Bin Mohamed, A. Selamat, and A. B. Hossain, “A Review of Recurrent Neural Network Based Camera Localization for Indoor Environments,” *IEEE Access*, vol. 11, pp. 43985–44009, 2023, doi: 10.1109/ACCESS.2023.3272479.

[19] J. C. Platt, “Sequential Minimal Optimization: A Fast Algorithm for Training Support Vector Machines,” 1998.

[20] J. Cervantes, F. Garcia-Lamont, L. Rodríguez-Mazahua, and A. Lopez, “A comprehensive survey on support vector machine classification: Applications, challenges and trends,” *Neurocomputing*, vol. 408, pp. 189–215, Sep. 2020, doi: 10.1016/J.NEUCOM.2019.10.118.

[21] F. F. Şimşek and S. S. Durduran, “Açık kaynak kodlu Eo-learn kütüphanesi ve çok zamanlı Sentinel-2 görüntüleri ile tarımsal ürün sınıflandırması,” *Journal of Geodesy and Geoinformation*, vol. 10, no. 1, pp. 45–62, May 2023, doi: 10.9733/jgg.2023r0004.t.

[22] A. Pektaş *et al.*, “Ağaç Tohum Algoritmasının Kümeleme Problemlerine Uygulanması,” 2022.

[23] H. HIZLI, Ç. TAKMA, and Ş. ERGÜL, “Application of Classification and Regression Tree (CRT) Method for Predicting the Some Environmental Factors Affecting Weaning Weight of Awassi Lamb,” *Journal of Agricultural Faculty of Gaziosmanpasa University*, Dec. 2022, doi: 10.55507/gopzfd.1169234.

[24] E. TURHAN, “Ardışık Gözlem İstasyonu Verileri ile Çoklu Doğrusal Olmayan Regresyon Yöntemi Kullanılarak Akım Tahmin Çalışması,” *Karadeniz Fen Bilimleri Dergisi*, vol. 12, no. 2, pp. 931–945, Dec. 2022, doi: 10.31466/kfbd.1175582.

[25] O. Eydemir and S. Boran, “Ayçiçeği Üretim Miktarının ANFIS Metodu ile Tahmini,” 2022.

[26] B. Koyuncu1 and M. Gök2, “BİTKİ ISLAHINDA GENOTİP VERİM DEĞERİNİN REGRESYON YÖNTEMLERİ İLE TAHMİNİ,” *BAHÇE*, vol. 48, no. 2, pp. 79–85, 2019.

[27] O. YAMAN and T. TUNCER, “Bitkilerdeki Yaprak Hastalığı Tespiti için Derin Özellik Çıkarma ve Makine Öğrenmesi Yöntemi,” *Fırat Üniversitesi Mühendislik Bilimleri Dergisi*, vol. 34, no. 1, pp. 123–132, Mar. 2022, doi: 10.35234/fumbd.982348.

[28] G. GÜNGÖR, A. AKÇAY, S. SARIÖZKAN, and E. ÇELİK, “Buzağı Hastalıkları Üzerine Etkili Faktörlerin Sınıflandırılmasında Yapay Sinir Ağları, Rassal Orman Algoritması ve Lojistik Regresyon Analizi Performanslarının Karşılaştırılması,” *Erciyes Üniversitesi*

Veteriner Fakültesi Dergisi, vol. 19, no. 2, pp. 94–100, Aug. 2022, doi: 10.32707/ercivet.1142552.

[29] S. Kiliçarslan, B. Onyedi Eylül Üniversitesi, and M. ve Doğa Bilimleri Fakültesi, "Kuru Üzüm Tanelerinin Sınıflandırılması için Hibrit Bir Yaklaşım," 2022.

[30] H. Çetiner, "Comparison of Different Machine Learning Methods for Estimating Agricultural Products," 2022.

[31] B. Babayigit *et al.*, "Comparison of Machine Learning Regression Models for the Prediction of Soil Moisture with the use of Internet of Things Irrigation System Data Nesnelerin İnterneti Sulama Sistemi Verileri Kullanılarak Toprak Neminin Tahmini için Makine Öğrenimi Regresyon Modellerinin Karşılaştırılması," 2021.

[32] T. ENSARİ, D. C. ARMAH, A. E. BALSEVER, and M. DAĞTEKİN, "Görüntü Tabanlı Dijital Bitki Fenotiplemesi için Konvolüsyonel Sinir Ağları," *European Journal of Science and Technology*, pp. 338–342, Aug. 2020, doi: 10.31590/ejosat.780087.

[33] İ. KACAR and C. KORKMAZ, "Prediction of agricultural drying using multi-layer perceptron network, long short term memory network and regression methods," *Gümüşhane Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, Oct. 2022, doi: 10.17714/gumusfenbil.1110463.

[34] M. Üstüner and F. Balık Şanlı, "Çok zamanlı polarimetrik SAR verileri ile tarımsal ürünlerin sınıflandırılması," *Journal of Geodesy and Geoinformation*, Dec. 2019, doi: 10.9733/jgg.2020r0001.t.

[35] E. EFE and U. ALGANCI, "Çok zamanlı Sentinel 2 uydu görüntüleri ve makine öğrenmesi tabanlı algoritmalar ile arazi örtüsü değişiminin belirlenmesi," *Geomatik*, vol. 8, no. 1, pp. 27–34, Apr. 2023, doi: 10.29128/geomatik.1092838.

[36] E. DANDIL, M. TURKAN, M. BOĞA, and K. K. ÇEVİK, "Daha Hızlı Bölgesel-Evrişimsel Sinir Ağları ile Sığır Yüzlerinin Tanınması," *Bilecik Şeyh Edebali Üniversitesi Fen Bilimleri Dergisi*, vol. 6, pp. 177–189, Sep. 2019, doi: 10.35193/bseufbd.592099.

[37] H. BİNGOL, "Derin Öğrenme Modellerinde Komşuluk Bileşen Analizi Yöntemi Kullanarak Çiçek Görüntülerinin Sınıflandırılması," *Fırat Üniversitesi Mühendislik Bilimleri Dergisi*, vol. 34, no. 1, pp. 439–447, Mar. 2022, doi: 10.35234/fumbd.1053501.

[38] Z. B. ERSOY, U. OKKAN, and O. FISTIKOĞLU, "DİNAMİK SU BÜTÇESİ MODELİNE DESTEK VEKTÖR REGRESYONU ENTEGRASYONU," *Uludağ University Journal of The Faculty of Engineering*, pp. 237–250, Mar. 2022, doi: 10.17482/uumfd.1009558.

[39] T. Uzaktan Algılama ve CBS Dergisi, M. Üstüner, S. Abdikan, G. Bilgin, and F. Balık Şanlı, "Turkish Journal of Remote Sensing and GIS Hafif Gradyan Artırma Makineleri ile Tarımsal Ürünlerin Sınıflandırılması Crop Classification Using Light Gradient Boosting Machines," 2020.

[40] N. ÖZÇANKAYA and M. BATUR, "İzmir Orman Bölge Müdürlüğü fıstıkçamı (Pinus pinea L.) meşcerelerinde kütük çapı–göğüs çapı ilişkisinin modellenmesi," *Ormancılık Araştırma Dergisi*, vol. 9, no. 1, pp. 44–60, Jun. 2022, doi: 10.17568/ogmoad.952271.

[41] İ. ÇINAR, M. KOKLU, and Ş. TAŞDEMİR, "Kuru Üzüm Tanelerinin Makine Görüşü ve Yapay Zeka Yöntemleri Kullanılarak Sınıflandırılması," *Gazi Journal of Engineering Sciences*, vol. 6, no. 3, pp. 200–209, Dec. 2020, doi: 10.30855/gmbd.2020.03.03.

[42] F. A. Şenel, "Makine Öğrenmesi Algoritmaları Kullanılarak Kayısı İç Çekirdeklerinin Sınıflandırılması," 2020.

[43] P. Cihan, O. Kalıpsız, and E. Gökçe, "Computer-aided diagnosis in neonatal lambs," *Pamukkale University Journal of Engineering Sciences*, vol. 26, no. 2, pp. 385–391, 2020, doi: 10.5505/pajes.2019.51447.

[44] G. YÜKSEL and H. SÖKÜN, "EGE BÖLGESİ İÇİN DİNAMİK MOD AYRIŞTIRMASI İLE KURAKLIK ANALİZİ," *International Journal of 3D Printing Technologies and Digital Industry*, vol. 6, no. 1, pp. 54–61, Apr. 2022, doi: 10.46519/ij3dptdi.1025073.

[45] E. E. Başakın, Ö. EKMEKCİOĞLU, and M. Ozger, "Drought Analysis with Machine Learning Methods," *Pamukkale University Journal of Engineering Sciences*, vol. 25, no. 8, pp. 985–991, 2019, doi: 10.5505/pajes.2019.34392.

[46] A. Yavuzdoğan and E. Tanır Kayıkçı, "Ege Denizi'nde makine öğrenimi yöntemleri ile anlık deniz seviyesi değişimlerinin tahmini," *Journal of Geodesy and Geoinformation*, vol. 8, no. 2, pp. 84–103, Nov. 2021, doi: 10.9733/jgg.2021r0007.t.

[47] M. A. Günen, "Fruit Detection from Apple Orchard Using Point Cloud Data," *El-Cezeri Journal of Science and Engineering*, vol. 9, no. 1, pp. 253–265, 2022, doi: 10.31202/ecjse.962269.

[48] A. Aydınoğlu and G. Altürk, "Heyelan Duyarlılık Haritalarının İstatistik ve Makine Öğrenmesi Teknikleri Kullanılarak Üretilmesi: Taşlıdere Havzası Örneği (Rize)," *Coğrafya Dergisi / Journal of Geography*, vol. 0, no. 43, pp. 159–176, Dec. 2021, doi: 10.26650/jgeog2021-814561.

[49] İ. Kacar and C. Korkmaz, "N25P5K5 Türü Yeni Nesil Bir Bitki Besininin Kuruma Kinetiğinin Regresyon ve Makine Öğrenme Yöntemleri ile Belirlenmesi," 2022.

[50] K. DEMİR, "Makine Öğrenmesi Tabanlı Tüm Avrupa için Tarımsal Ekim Planlaması," *European Journal of Science and Technology*, Feb. 2021, doi: 10.31590/ejosat.822785.

[51] H. DUYSAK, U. ÖZKAYA, and E. YİĞİT, "Makine Öğrenmesi Yöntemleri ile Tahıl Yüzey Sınıflaması," *European Journal of Science and Technology*, Oct. 2020, doi: 10.31590/ejosat.802719.

[52] G. COŞKUN, Ö. ŞAHİN, İ. A. OZKAN, and İ. AYTEKİN, "Siyah Alaca Sığırlarda Farklı Büyüme ve Gelişme Dönemlerindeki Vücut Ölçülerinden Canlı Ağırlık Tahmininde Kullanılan Veri Madenciliği Algoritmalarının Karşılaştırılması," *Ziraat Mühendisliği*, no. 375, pp. 37–46, Sep. 2022, doi: 10.33724/zm.1092837.

[53] H. C. BAYRAKÇI, R. S. ÇİÇEKDEMİR, and M. ÖZKAHRAMAN, "Tarım Arazilerinde Harcanan Su Miktarını Yapay Zekâ Teknikleri Kullanarak Belirlenmesi1," *Düzce Üniversitesi Bilim ve Teknoloji Dergisi*, vol. 9, no. 6, pp. 237–250, Dec. 2021, doi: 10.29130/dubited.1015690.

[54] Ş. Kilinçarslan, Y. Şimşek Türker, and M. İnce, "Temas Açısı Değerlerine Bağlı Ağaç Türlerinin Farklı Sınıflandırma Yöntemleri İle Tahmini," *Bartın Orman Fakültesi Dergisi*, vol. 22, no. 3, pp. 861–870, 2020, doi: 10.24011/barofd.697098.

[55] G. P. Baitu, O. A. A. Gadalla, and Y. B. Öztekin, "Traditional Machine Learning-Based Classification of Cashew Kernels Using Colour Features," *Journal of Tekirdag Agricultural Faculty*, vol. 20, no. 1, pp. 115–124, Jan. 2023, doi: 10.33462/jotaf.1100782.

[56] F. KURTULMUŞ, A. POLAT, and N. İZLİ, "Yapay Sinir Ağları Kullanarak Kayısının Farklı Kurutma Yöntemleriyle Kurutulmasında Kuruma Hızı Ve Nem İçeriği Parametrelerinin Modellenmesi," *ÇOMÜ Ziraat Fakültesi Dergisi*, vol. 8, no. 2, pp. 261–269, Dec. 2020, doi: 10.33202/comuagri.733166.

[57] H. Yıldırım and M. Karaatlı, “Yapay Sinir Ağları NARX Modeli ile Elma Üretim Miktarının Öngörülmesi,” 2022. [Online]. Available: http://www.fao.org/faostat/en/#data/QC/visualize,
[58] Y. C. Çolak and M. Özdemir, “ÇAYKUR İşletmeleri için Bölge Müdürlüğü Seçim Önerisi,” 2021. [Online]. Available: https://orcid.org/0000-
[59] T. Uzaktan Algılama ve CBS Dergisi, M. Üstüner, S. Abdikan, G. Bilgin, and F. Balık Şanlı, “Hafif Gradyan Artırma Makineleri ile Tarımsal Ürünlerin Sınıflandırılması,” 2020.

EXPLORING THE IMPACT OF TRANSFORMERS AND STATE SPACE MODELS IN LANGUAGE AND SEQUENCE PROCESSING

Tuna TOPAÇ[1], Selçuk YAZAR[2], Erdem UÇAR[1]

[1] Trakya University, Faculty of Applied Sciences, Management Information Systems Department, Edirne, Turkey
[2] Kırklareli University, Engineering Faculty, Department of Software Engineering, Kırklareli, Turkey

Corresponding author e-mail: tunatopac72@trakya.edu.tr

ABSTRACT

In the field of machine learning, the sequential processing technique used especially in natural language processing and the attention mechanism, which has become popular recently, direct the sector and the world of informatics. These two techniques have become even more important with their transformer and space state model architectures. Space state models also appear in finance, signal processing, and control systems. The strong prediction of the converter architecture and its resistance to long-term dependency when used, as well as the dynamic operation and speed of the space state models depending on the variables of the system, play a major role in this regard. Although these architectures are important in the field of natural language processing, they have also been used in science where sequential processing is important, such as bioinformatics. In this study, technical information about the architectures and the studies in the literature are given by comparing these two architectures, which are used in many fields.

Keywords: Machine Learning, Transformers, Space State Models, Sequential Processing

INTRODUCTION

Natural Language Processing (NLP) stands as a pivotal technology in the realm of computer science and artificial intelligence, aiming to bridge the communication gap between humans and machines by enabling computers to evaluate, interpret, and generate human language. Its significance spans various fields, underlining its versatility and critical role in advancing human-computer interaction. In the domain of natural language understanding, NLP facilitates the comprehension of complex language structures, allowing for the extraction of insights and actions, which is essential for artificial intelligence applications. This understanding is foundational for tasks such as text classification, named entity recognition, and language modeling, showcasing NLP's ability to parse and make sense of human language in a way that computers can process [1]. Machine translation exemplifies another vital application of NLP, breaking language barriers by translating text or speech from one language to another. This capability not only enhances communication across different language speakers but also supports global accessibility of information, making it indispensable in today's interconnected world [2], [3]. NLP is crucial for, healthcare, finance, and agriculture, among other fields, demonstrating its broad utility in understanding and responding to human and system interactions [4]. Overall, the significance of NLP across natural language understanding, machine translation, and sentiment analysis underscores its role in automating and enhancing interactions between humans and machines. By processing and generating human language, NLP not only facilitates more natural communication with computers but also supports decision-making and strategy development across diverse sectors[5].

Recent advancements in language processing, powered by deep learning, have been significantly influenced by the introduction and evolution of Transformer models. These models, originally developed for natural language processing (NLP), have revolutionized the field by leveraging self-attention mechanisms to process variable-length input sequences in parallel, capturing long-range dependencies more effectively than previous architectures[6]. This capability has led to their dominance in almost all NLP tasks, despite occasional errors that necessitate ongoing refinement and adaptation in industrial applications. Transformers have not only excelled in traditional NLP tasks but have also found applications in bioinformatics, particularly in proteomics, where they are used to predict protein properties from sequenced proteins, bridging the gap between the vast number of sequenced proteins and those with known properties[7], [8]. This application is part of a broader computational revolution in biology, where Transformer models are used to improve predictions and understandings of protein characteristics, including post-translational modifications. Beyond biology, Transformer models have been adapted for remote sensing image analysis, replacing conventional convolution operators with self-attention mechanisms to capture long-range dependencies in very high-resolution (VHR), hyperspectral (HSI), and synthetic aperture radar (SAR) imagery[9], [10]. This adaptation underscores the versatility of Transformer models across different domains. In the clinical domain, Transformer-based models have been applied to extract and structure information from radiology reports, outperforming previous deep learning models based on artificial neural networks (ANN) and convolutional neural networks (CNN)[11]. Additionally, the development of specialized models like TIS Transformer demonstrates the potential of deep learning techniques, originally designed for NLP, to learn the semantics of biological processes such as translation initiation site determination, further advancing our understanding of the proteome[12]. Moreover, the hardware acceleration of Transformer models addresses the computational challenges posed by their large size and the intensive memory requirements, showcasing significant improvements in latency and energy consumption over traditional deep neural network accelerators. This technological advancement supports the broader adoption and application of Transformer models across various fields. Finally, the transformative potential of Transformer language models (TLMs) extends to business and societal applications, where they are poised to enable new research topics and applications in information systems (IS), offering superior performance for a wide range of tasks and applications. This broad applicability and ongoing innovation underscore the significant impact of Transformer models on the field of deep learning and language processing.

Encoder-decoder architectures paired with attention mechanisms, as exemplified in Transformer models, and State Space Models (SSMs) represent two distinct methodologies for modeling sequential data. The former is composed of an encoder and a decoder that operate by processing input sequences in a sequential manner and generating output sequences with concurrent attention mechanisms, rendering them well-suited for applications such as machine translation. Conversely, SSMs explicitly characterize temporal dependencies through the formulation of hidden state evolution equations, showcasing proficiency in tasks like time-series analysis. Encoder-decoder architectures center on the acquisition of contextual information and the independent processing of input-output pairs, whereas SSMs prioritize the representation of temporal dynamics and sequential dependencies, often necessitating training techniques such as maximum likelihood estimation or Bayesian inference. These disparities underscore the unique capabilities of each approach in managing various forms of sequential data and diverse application domains. General features of the two architectures are shown in Figure 1.

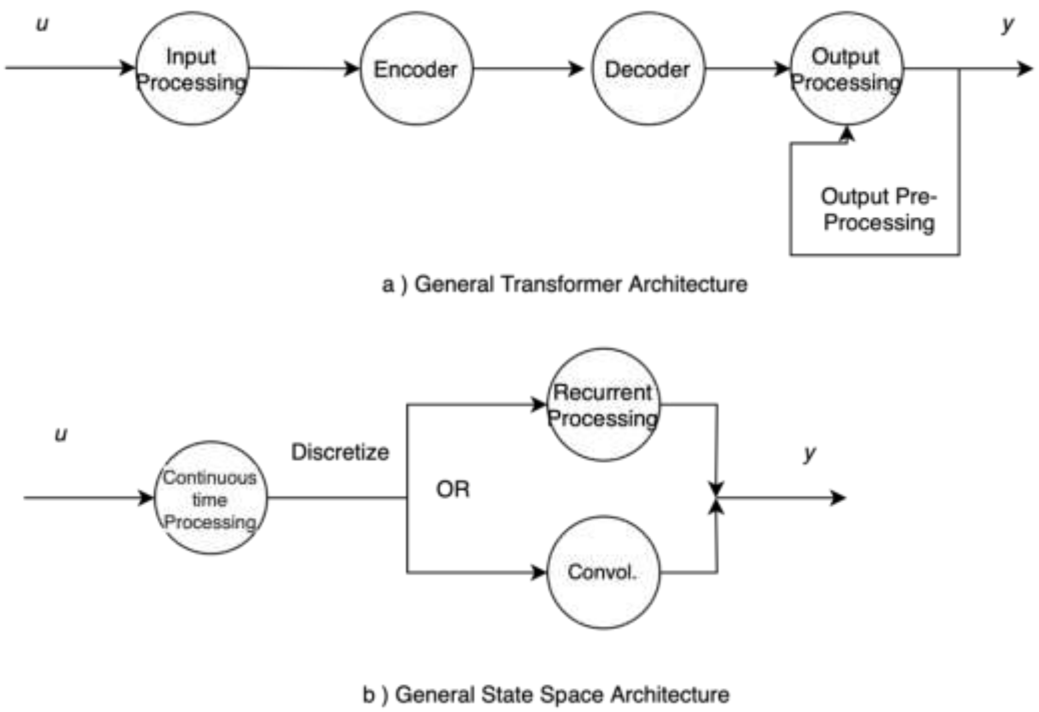


Figure 1. General structures of Transformers and State Space Models

BACKGROUND

In language processing, powered by deep learning, have seen significant contributions from the development and application of State Space Models (SSMs). These models have demonstrated state-of-the-art sequence modeling performance, challenging traditional sequence models like RNNs, CNNs, and even Transformers in some aspects. The introduction of the S4 model, which utilizes a HiPPO matrix for effectively managing long-range dependencies, marked a pivotal advancement in the use of SSMs for deep learning layers, showcasing their potential in handling complex tasks involving temporal sequences [13]. Despite their linear scalability with sequence length, which is a notable advantage over the quadratic scaling of Transformers, SSMs initially faced challenges in hardware utilization and expressivity in language modeling tasks [14]. To bridge the expressivity gap between SSMs and attention mechanisms in language modeling, new SSM layers like H3 have been proposed. These layers are designed to enhance the model's ability to recall earlier tokens and compare tokens across sequences, thereby improving performance on synthetic language tasks and coming close to matching Transformers on benchmarks like OpenWebText[15]. Furthermore, advancements in training efficiency, such as the introduction of FlashConv, have significantly improved the speed of SSM-based models, enabling them to outperform Transformers in certain scenarios [16]. Deep learning's impact on natural language processing (NLP) extends beyond SSMs, with neural network-based methods setting new performance benchmarks across a range of tasks, including speech recognition, language modeling, and semantic parsing [17]. The integration of deep variational Bayes filters and probabilistic modeling with SSMs has further enhanced the ability to learn complex, latent Markovian state space models, enabling unsupervised learning and identification of intricate patterns in data. Moreover, the exploration of deep neural network language models (DNN LMs) has shown promising improvements over traditional single-layer neural network language models (NNLMs), capturing higher-level discriminative information that contributes to more effective language models [18]. End-to-end models based on deep neural networks, including recurrent neural networks (RNNs) and attention-based encoder-decoder RNNs, have also gained popularity for their ability to process spoken language without the need for time alignment between input and output sequences, presenting a viable alternative to hybrid HMM-DNN systems [19]. In summary, the recent advancements in language processing enabled by deep learning, particularly through the development and application of state space models, have significantly pushed the boundaries of what's possible in NLP, offering improvements in efficiency, expressivity, and overall performance across a variety of tasks [20].

The advancements in sequence modeling through the introduction of the Mamba and Jamba algorithms, as inferred from the provided contexts, focus on addressing the limitations of traditional State Space Models (SSMs) and enhancing their performance in various sequence modeling tasks. One significant improvement is the development of models that efficiently handle long-form video understanding by selectively focusing on informative image tokens, thereby enhancing accuracy and reducing memory requirements. This approach, exemplified by the Selective S4 (S5) model, demonstrates a novel way of adaptively selecting image tokens to model long-term spatiotemporal dependencies more efficiently and accurately[21]. Similarly, advancements in language modeling have been made by addressing the expressivity gap between SSMs and attention mechanisms, with new SSM layers designed to recall earlier tokens and compare tokens across sequences, thereby closely matching the performance of attention-based models [22]. Further, the integration of Hamiltonian Monte Carlo (HMC) sampling with Sequential Monte Carlo (SMC) methods in nonlinear SSMs represents a significant leap in reducing model complexity and improving the adaptability to the local geometry of latent space. Additionally, the Sparse Modular Activation (SMA) mechanism introduces a way to dynamically activate sub-modules in neural networks, optimizing computation and memory usage during sequence modeling [23]. Moreover, the application of SSMs to machine translation highlighted the necessity of incorporating attention mechanisms to address S4's limitations in summarizing full source sentence. These advancements collectively underscore a trend towards more efficient, accurate, and flexible sequence modeling techniques, potentially indicative of the theoretical contributions of the Mamba and Jamba algorithms in enhancing selective state space models.

COMPARATIVE ANALYSIS

Transformers and State Space Models (SSMs) have been pivotal in advancing sequential modeling, each demonstrating unique strengths and limitations across various tasks. Transformers, renowned for their self-attention mechanism, have significantly impacted fields like speech recognition and natural language processing (NLP), outperforming Recurrent Neural Networks (RNNs) in accuracy and efficiency for sequence-to-sequence tasks such as automatic speech recognition. Their ability to approximate sequential relationships has been theoretically validated, showcasing their adaptability to different types of sequence modeling applications[24]. However, the quadratic computational cost associated with their attention mechanism poses limitations for processing long sequences [13]. On the other hand, SSMs, originally designed for continuous signals, excel in modeling long-range dependencies with sub quadratic runtime complexity, making them efficient for long sequences [25], [26]. Despite their scalability and efficiency, SSMs have struggled to match the performance of Transformers in language modeling tasks due to challenges in recalling earlier tokens and comparing tokens across sequences [27]. This gap is attributed to SSMs' underperformance in tasks requiring intricate local information processing, a domain where Transformers excel [28].

Recent advancements have aimed at bridging the gap between these models. The introduction of hybrid models, such as the Block-State Transformer (BST) and SPADE, combines the strengths of both SSMs and Transformers, leveraging SSMs for efficient long-range contextualization and Transformers for local sequence representation. These hybrid models have shown promising results, outperforming traditional Transformer architectures in language modeling perplexity and demonstrating superior speed and efficiency. Furthermore, innovations like FlashConv and the H3 layer have improved SSMs' hardware utilization and their ability to recall and compare tokens, narrowing the performance gap with Transformers in language modeling tasks. In summary, while Transformers excel in processing complex local information with high accuracy, their efficiency diminishes with longer sequences. SSMs, optimized for long sequences, have evolved through hybrid models and algorithmic improvements, offering competitive alternatives that combine the best of both worlds.

When the characteristics of Transformers and SSMs are compared, the main areas of use are similar. However, the tokenization approach, which is frequently used in natural language processing, is not used in SSMs, which may be an advantage here. However, although SSMs were not originally designed for natural language processing, they are quite successful in sequential data processing. The general characteristics of the two methods are shown in Table 1.

Table 1. characteristics of the Transformers and State Space Models

Transformers	State Space Models
Designed for sequential data	Designed for modeling dynamic systems
Self-attention mechanism	Markovian or non-Markovian dependencies
Parallel computation	Sequential computation
Effective for long-range dependencies	Effective for capturing system dynamics
Requires tokenization of input data	No explicit tokenization necessary
Commonly used in NLP tasks	Widely used in control systems, finance, signal processing, etc.

DISCUSSION

In general, the areas where both methods are used are similar. However, the recently emerging strengths of SSMs are thought to offer potential, especially in the field of bioinformatics. Integrating State Space Models (SSMs) in sequential modeling within the bioinformatics domain can significantly enhance the analysis and prediction of biological sequences, leveraging the strengths of SSMs in handling long-range dependencies and complex data structures. The S4 and S5 layers, as discussed by Jimmy Smith, Andrew Warrington, and Scott W. Linderman, demonstrate state-of-the-art performance in long-range sequence modeling by combining linear SSMs with deep learning, which could be particularly beneficial for modeling the sequential nature of genetic information and protein structures[29]. These models, especially the S5 layer with its multi-input, multi-output SSM, offer a promising framework for bioinformatics applications by efficiently handling sequences with long dependencies [30]. The Bayesian framework for filtering and parameter estimation, as explored in various studies, addresses the challenges of incomplete and noisy observations common in bioinformatics data, such as gene expression time series [31]. This approach, which avoids particle degeneracy and exploits low-rank tensor structures, could be adapted for more accurate modeling of biological systems [32]. Sequential Monte Carlo (SMC) sampling, highlighted by Mario V. Wüthrich, provides a powerful tool for solving non-linear and non-Gaussian state space models, which are prevalent in bioinformatics due to the complex and stochastic nature of biological processes. The integration of SMC methods with state-space models offers a robust framework for sequential analysis in bioinformatics [33]. Furthermore, the development of new sequential learning methods that exploit low-rank tensor-train decompositions for joint parameter and state estimation under the Bayesian framework, as discussed by Yiran Zhao and Tiangang Cui, introduces scalable function approximation tools that could significantly benefit bioinformatics applications by providing accurate and computationally efficient solutions [34].

REFERENCES

[1] T. Ul Haq ve N. Kapoor, "Comparative Analysis of Various Techniques of The Natural Language Processing", içinde *2023 Second International Conference on Electrical, Electronics, Information and Communication Technologies (ICEEICT)*, Trichirappalli, India: IEEE, Nis. 2023, ss. 01-07. doi: 10.1109/ICEEICT56924.2023.10157318.

[2] S. Pattanayak, *Pro deep learning with TensorFlow 2.0: a mathematical approach to advanced artificial intelligence in Python*, Second edition. New York, NY: Apress, 2023.

[3] K. P. Gunasekaran, "Exploring Sentiment Analysis Techniques in Natural Language Processing: A Comprehensive Review", 2023, doi: 10.48550/ARXIV.2305.14842.

[4] Abhay A. Dande ve Dr. M. A. Pund#, "A Review Study on Applications of Natural Language Processing", *IJSRSET*, ss. 122-126, Mar. 2023, doi: 10.32628/IJSRSET2310214.

[5] S. Chan, Ed., *Routledge encyclopedia of translation technology*, Second edition. Abingdon, Oxon: Routledge, 2023.

[6] A. Chandra, L. Tünnermann, T. Löfstedt, ve R. Gratz, "Transformer-based deep learning for predicting protein properties in the life sciences", *eLife*, c. 12, s. e82819, Oca. 2023, doi: 10.7554/eLife.82819.

[7] G.-L. Huang, A. Zaslavsky, S. W. Loke, A. Abkenar, A. Medvedev, ve A. Hassani, "Context-Aware Machine Learning for Intelligent Transportation Systems: A Survey", *IEEE Trans. Intell. Transport. Syst.*, c. 24, sy 1, ss. 17-36, Oca. 2023, doi: 10.1109/TITS.2022.3216462.

[8] A. A. Aleissaee *vd.*, "Transformers in Remote Sensing: A Survey", *Remote Sensing*, c. 15, sy 7, s. 1860, Mar. 2023, doi: 10.3390/rs15071860.

[9] N. Q. K. Le, "Leveraging transformers-based language models in proteome bioinformatics", *Proteomics*, c. 23, sy 23-24, s. 2300011, Ara. 2023, doi: 10.1002/pmic.202300011.

[10] R. Gruetzemacher ve D. Paradice, "Deep Transfer Learning & Beyond: Transformer Language Models in Information Systems Research", *ACM Comput. Surv.*, c. 54, sy 10s, ss. 1-35, Oca. 2022, doi: 10.1145/3505245.

[11] S. A. R. Moezzi, A. Ghaedi, M. Rahmanian, S. Z. Mousavi, ve A. Sami, "Application of Deep Learning in Generating Structured Radiology Reports: A Transformer-Based Technique", *J Digit Imaging*, c. 36, sy 1, ss. 80-90, Ağu. 2022, doi: 10.1007/s10278-022-00692-x.

[12] J. Clauwaert, Z. McVey, R. Gupta, ve G. Menschaert, "TIS Transformer: remapping the human proteome using deep learning", *NAR Genomics and Bioinformatics*, c. 5, sy 1, s. lqad021, Oca. 2023, doi: 10.1093/nargab/lqad021.

[13] D. Y. Fu, T. Dao, K. K. Saab, A. W. Thomas, A. Rudra, ve C. Ré, "Hungry Hungry Hippos: Towards Language Modeling with State Space Models", 2022, doi: 10.48550/ARXIV.2212.14052.

[14] A. Gu, A. Gupta, K. Goel, ve C. Ré, "On the Parameterization and Initialization of Diagonal State Space Models", 2022, doi: 10.48550/ARXIV.2206.11893.

[15] F. Schmidt ve T. Hofmann, "Deep state space models for unconditional word generation", içinde *Proceedings of the 32nd International Conference on Neural Information*

Processing Systems, içinde NIPS'18. Red Hook, NY, USA: Curran Associates Inc., 2018, ss. 6161-6171.

[16] M. Karl, M. Soelch, J. Bayer, ve P. van der Smagt, "Deep Variational Bayes Filters: Unsupervised Learning of State Space Models from Raw Data", 2016, doi: 10.48550/ARXIV.1605.06432.

[17] Z. Lu ve H. Li, "Recent Progress in Deep Learning for NLP", içinde *Proceedings of the 2016 Conference of the North American Chapter of the Association for Computational Linguistics: Tutorial Abstracts*, San Diego, California: Association for Computational Linguistics, 2016, ss. 11-13. doi: 10.18653/v1/N16-4004.

[18] A. Doerr *vd.*, "Probabilistic Recurrent State-Space Models", içinde *Proceedings of the 35th International Conference on Machine Learning*, J. Dy ve A. Krause, Ed., içinde Proceedings of Machine Learning Research, vol. 80. PMLR, Tem. 2018, ss. 1280-1289. [Çevrimiçi]. Erişim adresi: https://proceedings.mlr.press/v80/doerr18a.html

[19] E. Arisoy, T. N. Sainath, B. Kingsbury, ve B. Ramabhadran, "Deep neural network language models", içinde *Proceedings of the NAACL-HLT 2012 Workshop: Will We Ever Really Replace the N-Gram Model? On the Future of Language Modeling for HLT*, içinde WLM '12. USA: Association for Computational Linguistics, 2012, ss. 20-28.

[20] K. Audhkhasi *vd.*, "Recent progress in deep end-to-end models for spoken language processing", *IBM J. Res. & Dev.*, c. 61, sy 4/5, s. 2:1-2:10, Tem. 2017, doi: 10.1147/JRD.2017.2701207.

[21] A. Gu ve T. Dao, "Mamba: Linear-Time Sequence Modeling with Selective State Spaces", 2023, doi: 10.48550/ARXIV.2312.00752.

[22] O. Lieber *vd.*, "Jamba: A Hybrid Transformer-Mamba Language Model", 2024, doi: 10.48550/ARXIV.2403.19887.

[23] A. Buchholz, N. Chopin, ve P. E. Jacob, "Adaptive Tuning of Hamiltonian Monte Carlo Within Sequential Monte Carlo", *Bayesian Anal.*, c. 16, sy 3, Eyl. 2021, doi: 10.1214/20-BA1222.

[24] M. Fathi, J. Pilault, O. Firat, C. Pal, P.-L. Bacon, ve R. Goroshin, "Block-State Transformers", 2023, doi: 10.48550/ARXIV.2306.09539.

[25] Z. Liao, "Comparative analysis between application of transformer and recurrent neural network in speech recognition", *ACE*, c. 6, sy 1, ss. 524-529, Haz. 2023, doi: 10.54254/2755-2721/6/20230879.

[26] S. Zuo *vd.*, "Efficient Long Sequence Modeling via State Space Augmented Transformer", 2022, doi: 10.48550/ARXIV.2212.08136.

[27] N. Jiang, X. Liu, H. Liu, E. T. K. Lim, C.-W. Tan, ve J. Gu, "Beyond AI-powered context-aware services: the role of human–AI collaboration", *IMDS*, c. 123, sy 11, ss. 2771-2802, Ara. 2023, doi: 10.1108/IMDS-03-2022-0152.

[28] D. Jadeja, A. Khetri, A. Mittal, ve D. K. Vishwakarma, "Comparative Analysis of Transformer Models on WikiHow Dataset", içinde *2022 International Conference on Sustainable Computing and Data Communication Systems (ICSCDS)*, Erode, India: IEEE, Nis. 2022, ss. 655-658. doi: 10.1109/ICSCDS53736.2022.9761043.

[29] J. T. H. Smith, A. Warrington, ve S. W. Linderman, "Simplified State Space Layers for Sequence Modeling", 2022, doi: 10.48550/ARXIV.2208.04933.

[30] Y. Zhao ve T. Cui, "Tensor-train methods for sequential state and parameter learning in state-space models", 2023, doi: 10.48550/ARXIV.2301.09891.

[31] S. Särkkä ve L. Svensson, *Bayesian Filtering and Smoothing*, 2. bs. Cambridge University Press, 2023. doi: 10.1017/9781108917407.

[32] N. Chopin ve O. Papaspiliopoulos, "Introduction to State-Space Models", içinde *An Introduction to Sequential Monte Carlo*, içinde Springer Series in Statistics. , Cham: Springer International Publishing, 2020, ss. 11-25. doi: 10.1007/978-3-030-47845-2_2.

[33] N. Chopin ve O. Papaspiliopoulos, "SMC2, Sequential Inference in State-Space Models", içinde *An Introduction to Sequential Monte Carlo*, içinde Springer Series in Statistics. , Cham: Springer International Publishing, 2020, ss. 357-370. doi: 10.1007/978-3-030-47845-2_18.

[34] M. V. Wüthrich, "Sequential Monte Carlo Sampling for State Space Models", içinde *Robustness in Econometrics*, c. 692, V. Kreinovich, S. Sriboonchitta, ve V.-N. Huynh, Ed., içinde Studies in Computational Intelligence, vol. 692. , Cham: Springer International Publishing, 2017, ss. 25-50. doi: 10.1007/978-3-319-50742-2_2.

GEOLOGY AND GEOSTATISTICS OF AN OVERTHRUST STRUCTURE DEPOSIT: CASE OF THE IRON DEPOSIT OF CHAABET EL BALLOUT, NORTHEASTERN ALGERIA

Halimi FAHİMA[1]✉, Souadnia SABRİNA[2], Djabri Larbi[3],Boudries AMEL[4], benrabah Lilia[1]

[1]Department of Geology, Geology Research Laboratory- Annaba, Badji Mokhtar University, Annaba- Algeria,
[2] Department of Geology - Geology Research Laboratory – Annaba, Ferhat Abbas University, Setif1– Algeria,
[3] Department of Geology, water resources laboratory - Annaba, Badji Mokhtar University, Annaba- Algeria.
[4]Department of Agricultural Sciences, Geology Research Laboratory - Annaba, University of Skikda- Algeria,

✉***Corresponding Author Email*** ***:*** **fahima.halimi@univ-annaba.dz**

ABSTRACT

The estimation of ore resources in a deposit was traditionally done using conventional methods, but with the development of mining geostatistics from the 1970s onwards, geostatistical methods became favored. These geostatistical methods were applied to the Chaabat El Ballout deposit, for which a specific approach was proposed.The iron deposit of Chaabat El Ballout is located northeast of the city of Souk Ahras. This deposit belongs to the Sellaoua scales zone. Geological studies conducted as part of this thesis revealed a duplex structure. The estimation of iron resources was initially done using vertical geological cross-section methods by EREM. These resources were re-estimated using ordinary kriging geostatistical methods, taking into account the duplex structure. The variograms of the 'thickness of the mineralization-bearing horizon' (EHP) and the 'mineralization coefficient' (CM) show the same zonal anisotropy, where the major axis has a direction of N160° and the minor axis has a direction of N70°. Directional variograms in these two directions were adjusted using spherical models. The direction of the major axis of anisotropy corresponds to that of the thrust direction. The results of estimations using geostatistical methods were compared to those obtained by conventional methods. The large discrepancy between the resources estimated by conventional methods and those obtained by ordinary kriging is surely due to the discontinuity of the mineralization: The mineralization is regular in the horizontal plane but discontinuous in the vertical plane due to the duplex structure. The mineralization coefficient was calculated in the vertical plane and was taken into account for correcting the resource estimates. Thus, to optimize the estimation of ore resources in breccia-type deposits using geostatistical methods, it is necessary to consider mineralization coefficients that resemble a probability coefficient."

Keywords: Iron deposit, Geostatistical estimation, mineralization coefficient.

INTRODOCTION

The Chaabat El Balout iron deposit is located in the northeastern part of Algeria, 20 km northeast of the city of Souk Ahras (Fig. 1). It consists of several discontinuous bodies and is mined to meet the needs of the local industry. Hence, there is a need to characterize them geologically and to find optimal methods for resource/reserve estimation.

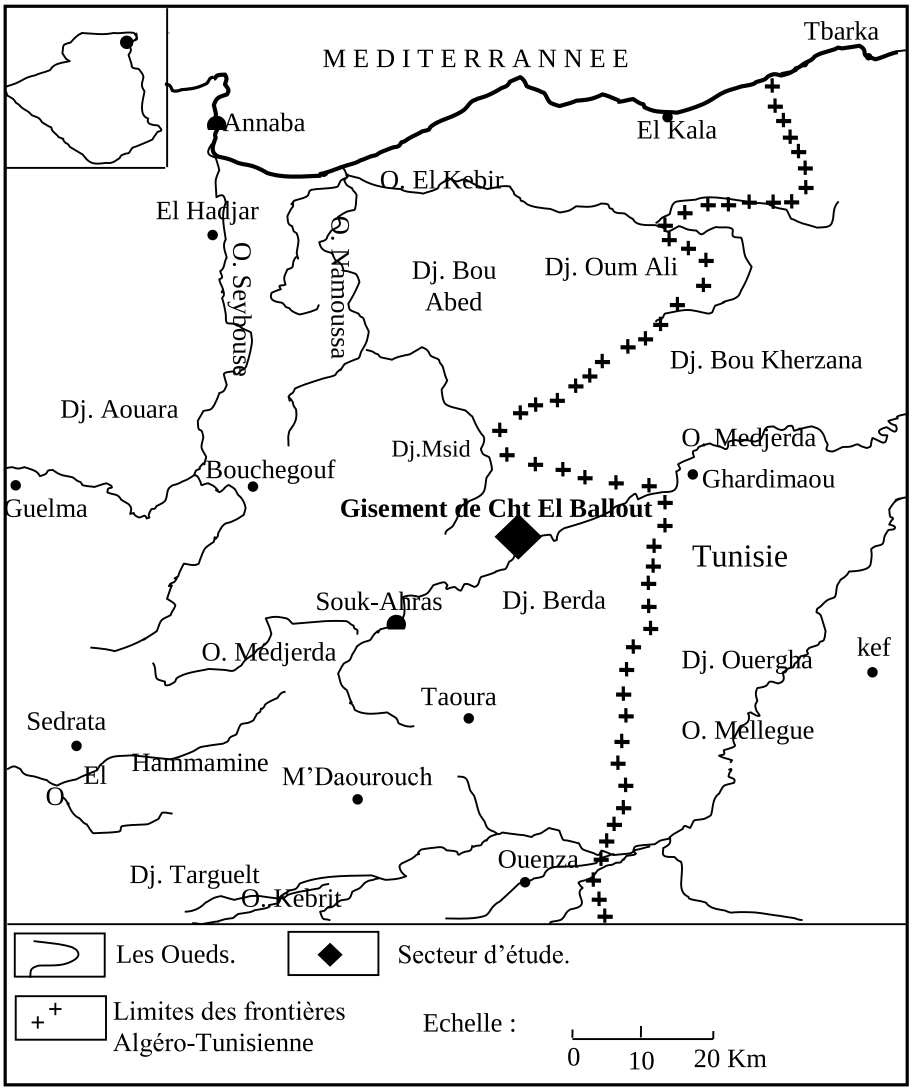


Figure 1. Geographical location of the Chaabet El Ballout iron deposit.

MATERIAL AND METHOD

The geological study of the region was conducted by David (1956), Kouzmine (1990), and Kriviakine et al. (1990).The deposit consists of upper Cretaceous age formations, lower Miocene and Quaternary. The ore body consists of a grinding zone with a thickness of 45 m and direction North West-South East (Fig. 2). This ore body plunges in depth with a dip of 30 ° to the southwest. The roof of this body is made of limestone while the wall is made up of sandstone and marl Miocene.

Geological cross-sections have been carried out, and among these sections (Fig.3), those conducted along the NW-SE direction show overlapping imbricate structures—duplexes (Boyer et al., 1982; Mitra et al., 1986).

These major thrusts generally correspond to detachment faults and are connected by several ramps—at least three—that define rock volumes (Fig. 3). In the Chaabet El Ballout deposit, the base of these duplex structures is composed of marls (Fig. 3). According to these cross-sections, the direction of the thrust is NW-SE, as proposed by Kriviakine et al. (1990). The variographic study was conducted using the Variowin software Pannatier (1996). The study was conducted on 240 core samples taken from 38 exploration holes. These samples were analyzed-On FeO, CaO, SiO_2.

The estimation of resources using the geostatistical method includes the following steps:

- Calculate the mineralization coefficient in the vertical plane.
- Calculate the thickness of the ore.
- Calculate the thickness of the mineralization-bearing horizon.
- Perform a 2D FeO variography.
- Kriging and, finally, resource/reserve estimation.

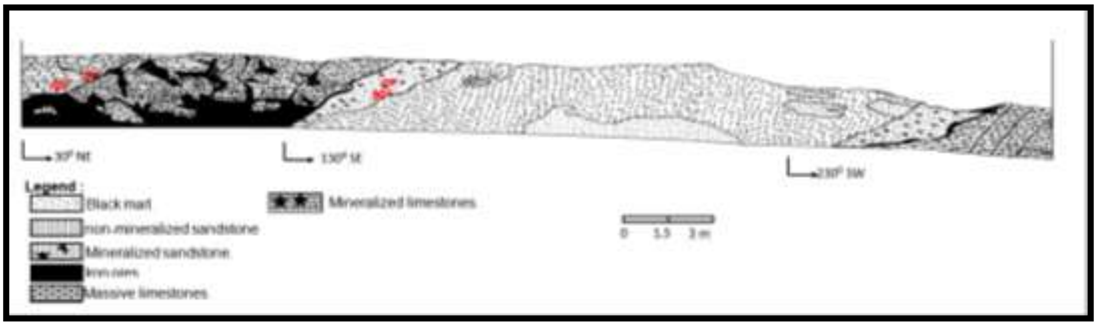


Figure 2. Geology career front bench N° :1020m.

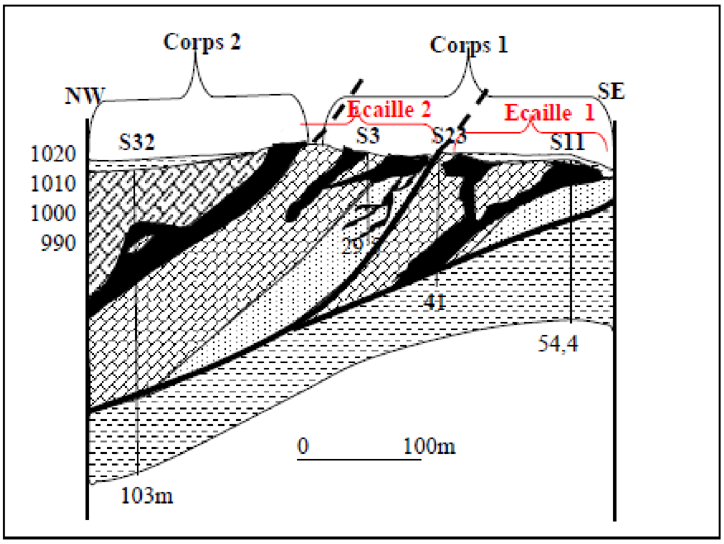


Figure 3. Geological cross-section along the NW-SE line

RESULTS AND DISCUSSION

The surface variograms (Fig. 4, 5) and directional experimental variograms of the regionalized variables—thickness of the mineralization-bearing horizon and mineralization coefficient (MC)—have been calculated and fitted (Fig. 6, 7) (Table 1).

The variograms of the thickness of the mineralization-bearing horizon(MBH) (Fig. 6) and the mineralization coefficient (Fig. 7) show zonal anisotropy, where the major axis is oriented N160° and the minor axis is oriented N70°.

Ordinary kriging was performed using the software Surfer08. Initially, the mean values of the regionalized variables EHP and CM, from 20x20m² surface blocks (Table 3), were kriged.

In the second phase, the mineralized thickness (MTH) was calculated using the following formula:
$MTH= MC_{krg}xTMTH_{krg}$

Where:

- MTH: The mineralized thickness of the (20x20m²) block;
- MCkrg: The kriged mineralization coefficient;
- TMTHkrg: The kriged total mineralized thickness.

- **Resource Estimation**

The estimation of iron ore resources for each block was done by multiplying the kriged variables MTH and CM by the block area (20x20m²) and the bulk density:
$Q_{minerai}$= MBH x CM x D x (20x20)
Given that MTH = MBH × CM, then Qore= MTH x D x (20x20)
Where:

- **D**: The bulk density.

At the Châabet El Ballout deposit, there are 5.6 million tonnes of mineralized rock containing 1.9 million tonnes of iron ore (Table 4).

- **Cartographie**

Les valeurs de l'épaisseur du minerai calculée des deux variables MBH. CM Krigées et leurs variances ont été cartographies (Figs. 8- 10)

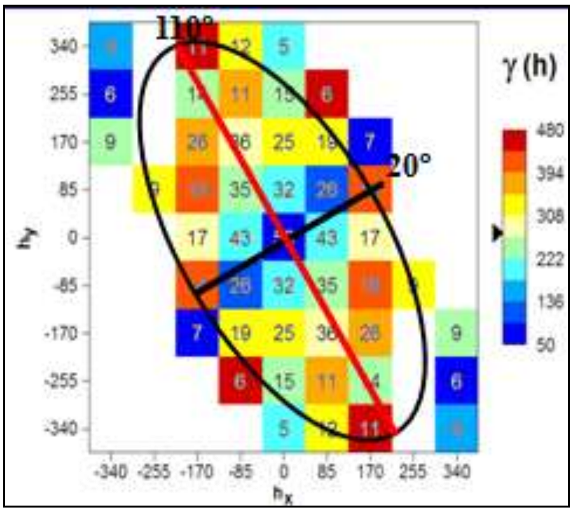


Figure 4. Surface variogram of the thickness of the mineralization-bearing horizon.

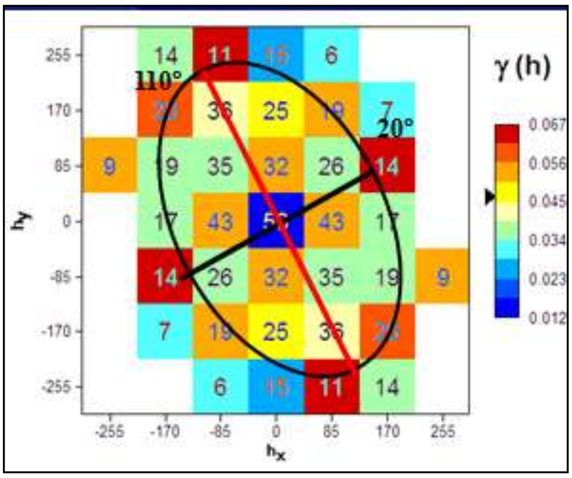


Figure 5. Surface variogram of the mineralization coefficient.

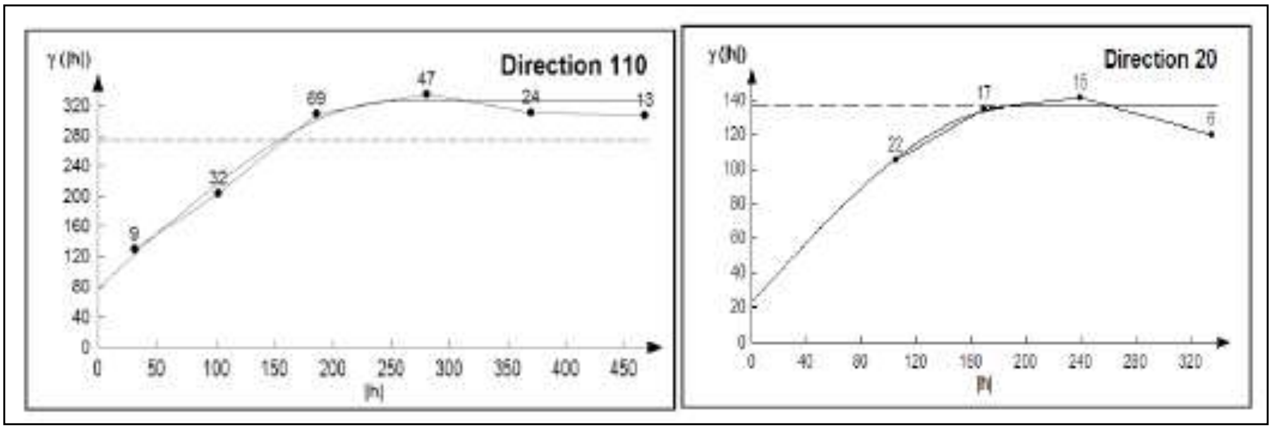


Figure 6. Directional experimental variograms of the thickness of the mineralization-bearing horizon and their fittings.

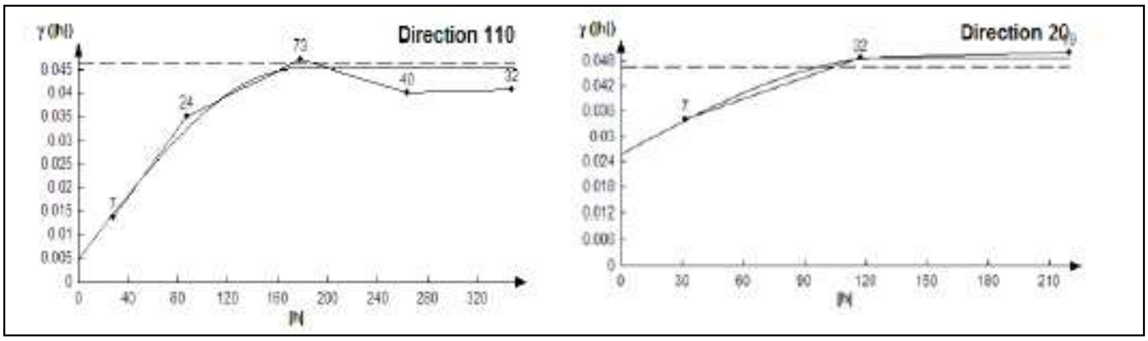


Figure7. Directional experimental variograms of the mineralization coefficient and their fittings.

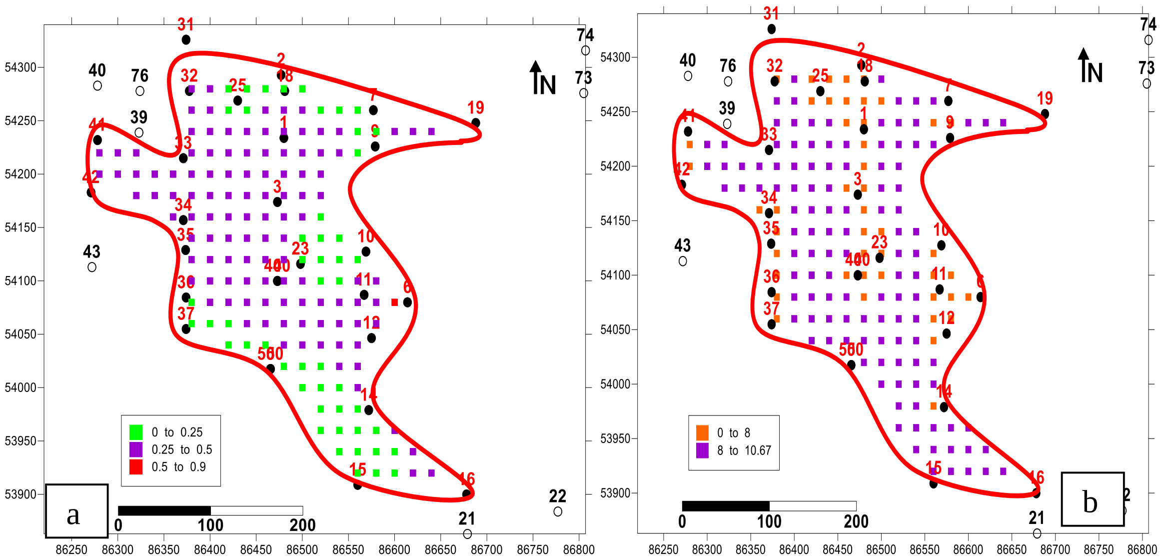


Figure 8. Map of the mineralization for (20X20) m^2 surface blocks. a: kriged, b: variance

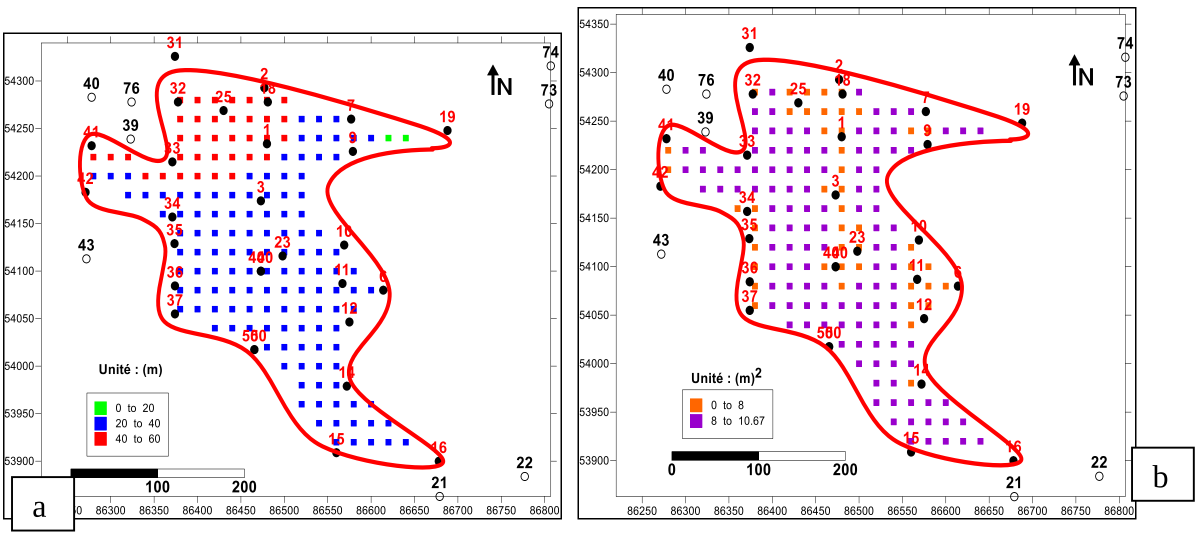


Figure 9. Map of the thicknesses of the mineralization-bearing horizon in 20x20 m² surface blocks. a: kriged, b: variance

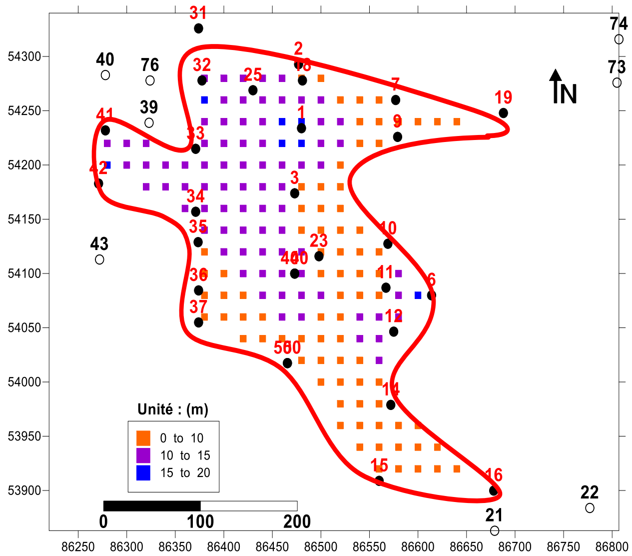


Figure 10. Map of the calculated mineralized thickness in 20x20 m² surface blocks.

Table 1. Variogram Adjustment Parameters of the thickness of the mineralization-bearing horizon.

Direction (°)	Range a (m)	Nugget effect C0	Sill C	Model
20	194	23.8	113.4	Spherical
110	257	78.39	249.2	Spherical

Table 2. Variogram Adjustment Parameters variograms of the mineralization coefficient.

Direction (°)	a (m)	C0	C	Model
20	132	0.026	0.023	Spherical
110	175	0.005	0.0405	Spherical

Table 3. Summary of iron resources and quantity of mineralized host rock.

MBH					MTH				
Classe des		N block	Mean (m)	Q mineralization-bearing	Classe de		N block	Mean	Q ore (t)
Min	Max				Min	Max			
0	20	2	18.40	35331.80	0	10	79	7.50	664012.90
20	40	127	32.30	3938800.20	10	15	79	12.76	1129491.80
40	60	36	42.92	1621814.70	15	20	7	16.62	130317.86
		Total=165	31.20	5595946.70			Total=165	12.29	1923822.60

CONCLUSIONS

The cross-sections show that the direction of the overlap is NW–SE, as proposed by Kriviakine et al. (1990). The grinding zone of the overlap (under the cover) may be confused with a stratiform layer.

The structural study of the deposit has clarified the orientation of the exploration profiles. These profiles should be perpendicular to the structure, thus oriented in the Southwest–Northeast direction.

The variograms for the thickness of the mineralization-bearing horizon (MBH) and the mineralization coefficient (MC) were fitted using spherical models. The direction of the major axis of anisotropy corresponds to the direction of the overlap.

At the Chaabet El Ballout deposit, there are 1.9 million tonnes of iron ore contained within 5.6 million tonnes of mineralized limestone, sandstone, and marl.

For optimizing the estimation of breccia ore resources using geostatistical methods, it is necessary to account for the mineralization coefficients, which are akin to a probability coefficient.

REFERENCES

BOYER, S. E. et ELLIOT, D., 1982. Thrust systems, Bull. Am. Ass. Petrol. Gol., 66, p. 1196-1230.

DAVID, L. 1956. Etude géologique des Monts de la Haute Medjerda. Service de la carte géologique de l'algérie, Alger, 289p.

KOUZMINE, G. 1992. Rapport final concernant les des travaux de recherche géologique du gisement de fer de Châabet El Ballout. 160p

KOUZMINE, G. 1992. Annexe N0 : 01 concernant le gisement de fer de Châabet El Ballout.102p.

KRIVIAKINE, B. KOVALENKO, E. VNOUTCHKOV, V. DERESKI, B. 1990. Rapport final sur les résultats de l'étude complémentaire des feuilles N0 : 77 Souk-Ahras, N0 :78 Oued Mougras, N0 : 100 M'Daourouche à l'échelle : 1/500000.

PANNATIER, Y. 1996. Variowin Software for Spatial Data Analysis in 2D. Springer verlag, pp91. ISBN 0-387-94679-9.

ENGINEERING PROPERTIES AND DETERIORATION OF IGNIMBRITES FROM WHICH KUŞKAYASI ROCK TOMBS WERE OPENED

Mustafa Korkanç

Niğde Ömer Halisdemir University, Faculty of Engineering, Department of Geological Engineering, Niğde, Turkey

Corresponding author e-mail: mkorkanc@ohu.edu.tr

ABSTRACT

The rock tombs subject to the research are in Karaatlı Town of Niğde Province Central District. There are 15 rock tombs located in the southwest of the town, 11 on the southern slope of the valley and 4 on the northern slope. The rock tombs, about which not much information can be found, were opened on ignimbrite rock masses, one of which is different from the others. The tombs located on the southern slope of the valley are more numerous, and the two-story large tomb is also located in this region. The rock tombs subject to the research continue to be subject to significant damage by treasure hunters, as well as deterioration caused by atmospheric effects. Block sample collection was carried out in order to determine the engineering properties of the ignimbrites where the tombs were found. Powder, fragment and core samples were prepared for various studies in the laboratory, and then the chemical, petrographic and geomechanical properties of the rock were determined. Due to the weak rock properties of tuff and ignimbrite type rocks and their easy excavation, there are many underground rock structures in Cappadocia region for different purposes. Rock tombs are also Late Roman Period structures and no research or definitions have been encountered other than their registration. In detailed research, the tombs opened in various sizes along the two slopes of the valley within the ignimbrites are empty and some of the tombs abandoned to neglect continue to be damaged by illegal treasure hunters every passing day. In order to provide a basis for studies to be carried out on the protection of such structures and their transfer to future generations, the engineering properties and deterioration of the rock in which the tombs were opened were tried to be determined within the scope of this study.

Keywords: Rock tomb, Ignimbrite, Petrographic properties, Engineering properties, Deterioration

INTRODUCTION

Rock tombs are known as tombs built for high-status individuals by many civilizations, in rock environments suitable for carving and processing. These structures are directly proportional to the development of the civilization. There are examples with very variable dimensions and aesthetic features in the world. No findings such as inscriptions or special reliefs that could help with dating were found in the examined rock tombs. There is no scientific study on the structures of the region so far. The engineering properties of the rock in which these tombs were opened constitute the subject of this study. Atmospheric weathering processes (such as freezing-thawing, wetting-drying and salt crystallization) primarily cause the crystals and minerals of the rock to decompose. As a result of the intense effects of these processes, different weathering types ranging from mm to m can develop. These effects can have more destructive results in rocks with low strength and high porosity properties such as tuff and ignimbrite. Especially in winter periods, such as Central Anatolia, the freezing of water and the expansion of its volume cause the existing intra-rock pores to increase (Fener and İnce, 2015). As this process progresses, cracks develop in the rock as a result of increasing pore volume, and integrity losses occur. In the studies on monuments and historical structures from past to present, weathering has been stated to be directly related to atmospheric actions (İpekoğlu et al. 2007; Korkanç 2013; Brimblecombe 2014), precipitation affecting the structure, groundwater (Sandrolini and Franzoni 2006; Fener and İnce 2015), petro-mechanics (Uchida et al. 2000) and hydraulics (Benavente et al. 2007) characteristics of the rock.

In this study, the rock material properties of the ignimbrites opened in the Karaatlı Rock Tombs, which are thought to belong to the Late Roman Period and about which no study has been done so far other than being registered, were investigated with laboratory and on-site non-destructive tests. It is expected that such studies will form the basis for future conservation studies.

MATERIALS AND METHOD

The rock tombs that are the subject of this study are in Karaatlı Town of Merkez District of Niğde Province. The rock tombs, which are also located in the southwest of the town, are located on two slopes of a valley. There are 15 tombs, 11 on the southern slope of the valley and 4 on the northern slope. There are kline inside these tombs that resemble each other. There are figures of dogs chasing mountain goats made with ochre in one tomb. There are holes like blind windows on the entrance doors of all of these rock tombs. Stylized human figures are carved in relief on the right and left of these doors (Niğde, 2007). The material properties of the rock unit where the tombs were opened were examined with Schmidt hardness hammer studies applied in the laboratory and on-site. Representative block samples were collected from the field for laboratory studies. Later, powder, fragment and core samples were prepared from these blocks according to the principles specified in ISRM (2007). According to the methods suggested in ISRM (2007) and TSE 699 (2009), dry and saturated unit volume weight, water absorption by weight, apparent porosity, capillary water absorption, P-wave velocity, Böhme wear repair and uniaxial pressure tests were performed in order to determine the geomechanical properties of the examined rock. In addition, the petrographic properties of the rock were determined by examining the prepared thin sections under optical microscope, and the main element contents were determined from the powder samples using the ICP-AES method.

The types of deterioration observed in the monument were classified according to the principles defined in ICOMOS (2008).

RESULTS AND DISCUSSIONS

Observations on rock tombs

Detailed field research has been conducted on the structure, which has no detailed research on it other than being registered in the literature. There are many underground structures in rocks such as tuff and ignimbrites, which are widespread in the Cappadocia region. These rocks were chosen because they are easy to process and on the surface. The rock tombs in the region have been opened in various sizes along the two slopes of the valley, and some of the tombs, which are empty and neglected, are still being destroyed by illegal treasure hunters. The rock tombs, about which not much information can be found, have been opened on ignimbrite masses, and one is different from the others. The other tombs have similar characteristics and are of similar sizes. The tombs on the eastern slope of the valley are more numerous, and the large tomb, which has two floors, is on the eastern slope.

Engineering properties of the ignimbrite samples

The main element contents of the examined samples were carried out with the ICP-AES method and the obtained results are presented in Table 1.

Table 1. Chemical composition of ignimbrites in the area where Kuşkayası rock tombs are located

Main element oxide (%)	SiO_2	Al_2O_3	Fe_2O_3	MgO	CaO	Na_2O	K_2O	TiO_2	P_2O_5	MnO	Cr_2O_3	LOI	Total
KA	74,16	13,43	1,30	0,23	1,79	3,57	3,85	0,20	0,06	0,05	<0,002	1,2	99,96

According to the obtained data, the SiO_2 contents of the ignimbrites collected from the region are relatively high. When examined in the total alkali-silicate diagram based on the chemical analysis data, it was determined that the ignimbrites were of rhyolitic composition.

Microscope examinations were made on two thin sections prepared on the collected ignimbrites sample and cracks and fractures were observed in these minerals where feldspar and plagioclase were observed as large phenocrysts, and opacifications were observed in places in the groundmass. For example, the ratio of phenocrysts in the first section is higher and their sizes are formed by relatively larger phenocrysts. The voids in the second section are relatively more than in the first section. The matrix ratio is higher than the grain ratio in both sections (Figure 1, Figure 2).

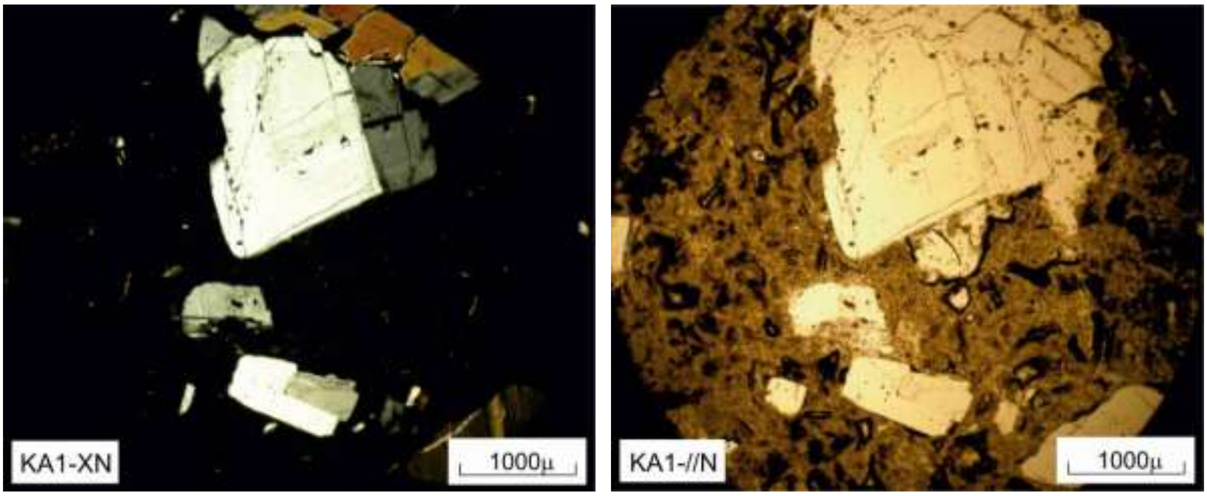


Figure 1. Microphotograph of sample no. KA a) Double-Nicol image, b) Single-Nicol image (magnification 4X)

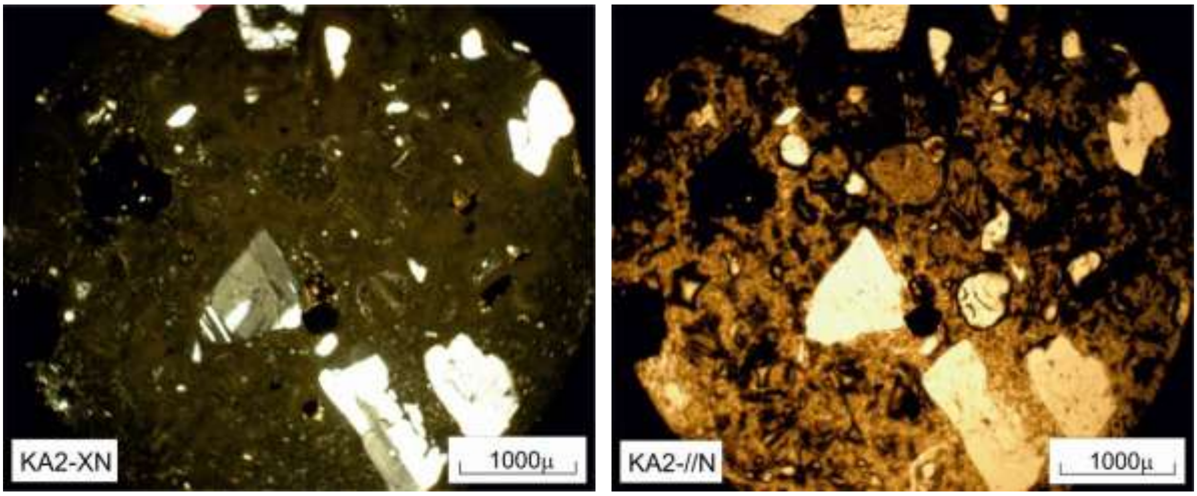


Figure 2. Another microphotograph of sample KA a) Double-Nicol image, b) Single-Nicol image (magnification 4X)

Opal-CT, Plagioclase (Plj.) and Feldspar (Feld.) minerals were observed in the examinations carried out by XRD method on powder samples prepared from blocks taken from the section where the rock tombs were excavated (Figure 3).

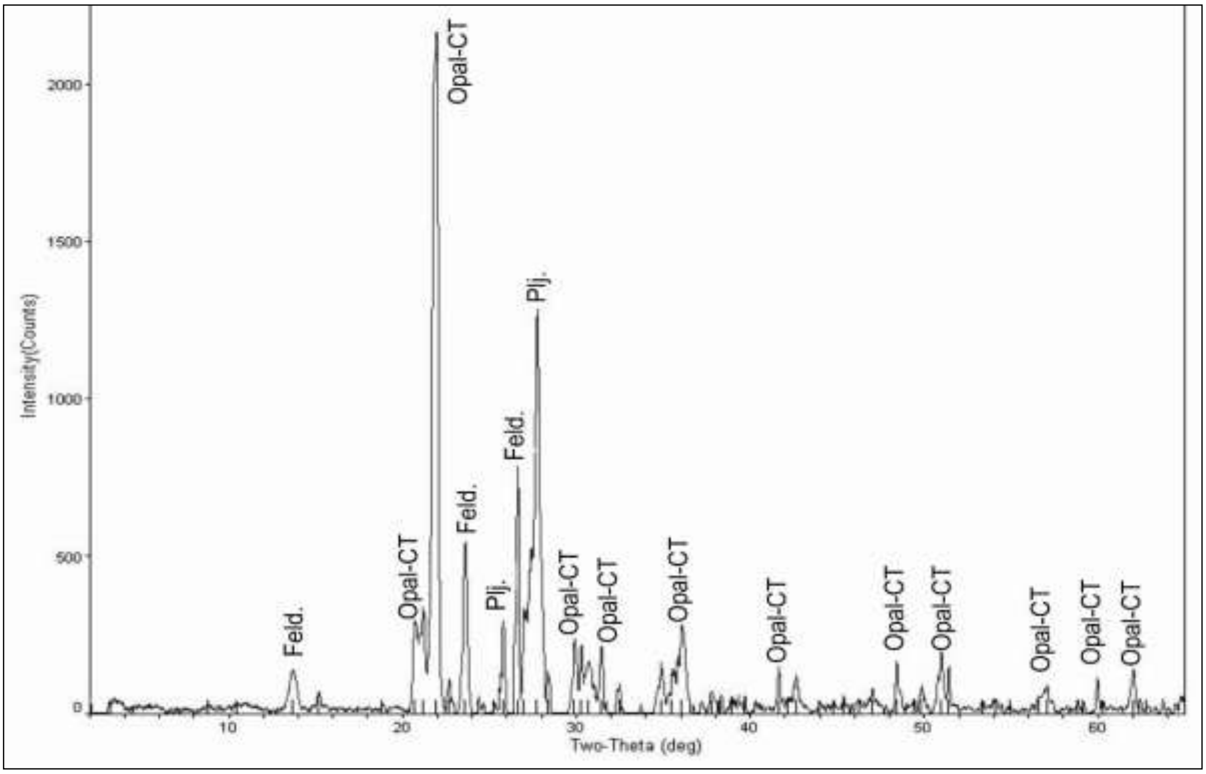


Figure 3. Diffractometer analysis results of the KA sample

In order to determine the engineering properties of the ignimbrites in the section where the rock tombs were opened, the data of dry and saturated unit weight, water absorption by weight, apparent porosity, P-wave velocity, stability index against dispersion in water as 2 cycles, capillary water absorption, Böhme surface abrasion and uniaxial compressive strength values and in-situ Schmidt hammer hardness measurements are presented below (Table 2 and Table 3).

Table 2. Engineering properties of the sample taken from the section where the rock tombs are located

Engineering feature	Number of Experiments	Min.	Max.	Average	S. Deviation
γ_d (kN/m^3)	5	17,84	18,25	18,09	0,18
γ_s (kN/m^3)	5	20,03	20,45	20,31	0,17
w_a (%)	5	11,85	12,65	12,23	0,30
n (%)	5	22,02	23,18	22,56	0,46
V_p (m/sn)	5	1833,56	2041,13	1941,58	76,09
Id_2 (%)	2	96,78	97,03	96,91	0,09
K x10^{-5} (cm^2/sn)	5	9,27	10,25	9,73	0,43
ΔV (cm^3/50 cm^2)	3	34,20	35,00	34,73	0,46
σc (MPa)	3	26,0	34,2	29,4	43

The ignimbrites examined were classified as rocks with “very low unit volume weight” according to NBG (1985). Again, according to NBG (1985), when classified in terms of porosity, they are classified as “rocks with very high porosity”. According to the classification made according to the sonic velocity values obtained according to ISRM (1978), they were classified as rocks with “very low seismic velocity”. When the examined samples were evaluated according to their uniaxial compressive strength values according to ISRM (1981), they are in the “moderately resistant rock” classes (Table 13).

The obtained dry and saturated unit weight and values are relatively high, and the water absorption values are relatively low compared to other ignimbrites. The P-wave velocity and the resistance index to dispersion in water values are also relatively high. The abrasion losses of the examined sample are high compared to other rocks such as marble and limestone, and their compressive strengths are relatively low. The L-type Schmidt hardness hammer rebound performed on the field grave samples is presented below (Table 3).

Table 3. Schmidt hammer hardness index values measured from different areas in the rock tombs section.

Schmidt Hammer Hardness Index, SHV (L-type)					
Measurement location	**Number of Measurements**	**Min.**	**Max.**	**Average**	**S. Deviation**
Entrance portal of rock tomb no. 1	10	42	55	52,9	6,60
Entrance portal of rock tomb no. 2	10	44	56	53,1	5,80
Base section of rock tomb no. 2	10	37	56	50,3	7,00
Entrance portal of rock tomb no. 3	10	41	55	52,5	4,85
Entrance portal of rock tomb no. 4	10	43	54	48,5	5,20
Entrance portal of rock tomb no. 5	10	42	55	53,4	3,25
Entrance portal of rock tomb no. 6	10	42	57	53,6	4,50
Entrance portal of rock tomb no. 7	10	40	55	48,2	5,70
Entrance portal of rock tomb no. 8 (decomposition partially active)	10	32	40	33,8	3,70
Entrance portal of rock tomb no. 1 on the western slope of the valley	10	47	55	54,2	2,80
Entrance portal of rock tomb no. 2 on the western slope of the valley	10	40	54	52,5	4,80

The ignimbrites in the entire area were evaluated as "hard rock" and "very hard rock" according to the classification made according to Beer (1967) (Table 3).

Deterioration and suggestions observed on the stones where the graves were opened

Almost all of the rock tombs in the region, except for one, have similar characteristics. They were opened in ignimbrites that also spread at the same levels in the field. Those on the eastern slope of the valley are more in number. Some of the tombs, on which no detailed study has been conducted to date, have been destroyed by illegal treasure hunters. There are no protection measures in the region. Algae and moss formations were observed on the outer side of some rock tombs. In the section where the large rock tomb is located, superficial losses developed in the area intersected by the vertical cooling crack. In general, with the effects of surface decomposition, the rock mass is partially effective in a few cm of the outer part of the surface. No significant effect was observed in the other inner sections. Destruction due to human effects (graffiti) is quite high in some tombs, especially by illegal treasure hunters. It is thought that there will be a tourism potential due to the different characteristics of the rock structures in the region, depending on the archaeological evaluation, cleaning and protection measures taken. Images of the problems obtained from the field surveys conducted in the area where the rock tombs are located are presented below (Figures 4 - 6)

Figure 4. View of the destruction in the tombs and kline where there are two-storey kline that are different from the others.

Figure 5. Falls due to the intersection of cracks, graffiti due to human effects and illegal excavation areas

Figure 6. Effects of biological deterioration (development of lichens, moss and flowering plants)

CONCLUSIONS

Many underground structures have been opened in rocks such as tuff and ignimbrites, which are common in the Cappadocia region, due to their easy processing and being on the surface. Rock tombs in the region have also been opened in various sizes along both slopes of the valley, and some of the tombs, which are empty and neglected, are still being destroyed by illegal treasure hunters. Surface collapses have been observed in areas intersecting with discontinuities and are open to deterioration due to atmospheric effects. The effects of biological deterioration and destruction due to human effects (graffiti) in the area are quite high, especially in some tombs by illegal treasure hunters.

REFERENCES

ASTM E494. 2010. Standard Practice for Measuring Ultrasonic Velocity in Materials. West Conshohocken: ASTM International.

Benavente D, Cueto N, Martínez-Martínez J, García del Cura MA, Cañaveras JC 2007. The influence of petrophysical properties on the salt weathering of porous building rocks, Environmental Geology, 52(2), 215-224.

Brimblecombe P. 2014. Environment and Architectural Stone, Stone in Architecture Properties, Durability eds. Siegesmund, S. and Snethlage, R., 317-348.

De Beer, J.H., 1967. Subjective classification of the hardness of rocks and the associated shear strength, Proc. 4th Reg. Cong. Afr. Soil Mech, Found Engineering, 396-398, Capctawn,

Fener, M., & İnce, İ. 2015. Effects of the freeze–thaw (F–T) cycle on the andesitic rocks (Sille-Konya/Turkey) used in construction building. Journal of African Earth Sciences, 109, 96-106.

ISRM, International Society for Rock Mechanics, 1978. Suggested Methods for the Quantitative Description of Discontinuities in Rock Masses. International Journal of Rock Mechanics and Mining Sciences & Geomechanics Abstracts, 15, 319-368.

ISRM International Society for Rock Mechanics, 1981. Rock characterization, testing and monitoring. ISRM Suggested methods. E.T. Brown (ed.), Pergamon Press, 211 pp.

ISRM, International Society for Rock Mechanics, 2007. The complete ISRM suggested methods for rock characterization, testing and monitoring: 1974–2006. In: Ulusay and Hudson (Eds.), Suggested methods prepared by the commission on testing methods, International Society for Rock Mechanics. ISRM Turkish National Group, Ankara, Turkey. 628 pp.

İpekoğlu, B., Böke, H., Çizer, Ö., 2007. Assessment of material use in relation to climate in historical buildings, Building and Environment, 42: 970-978.

ICOMOS-ISCS. 2008. International Council on Monument and Sites International Scientific Committee for Stone, Illustrated Glossary on Stone Deterioration Patterns. Champigny/Marne, France: Ateliers 30 Impresión.

Korkanç, M. 2013. Deterioration of different stones used in historical buildings within Nigde province, Cappadocia, Construction and Building Materials, 48: 789-803.

NBG. 1985. Engineering Geology and Rock Engineering, Handbook No. 2. 2nd ed. Trondheim, Norway: Norwegian Group for Rock Mechanics (NBG).

Niğde, 2007. Seçil Ofset, s. 120, İstanbul.

Sandrolini, F, Franzoni, E. 2006. An operative protocol for reliable measurements of moisture in porous materials of ancient buildings, Building and Environment, 41: 1372-1380.

TS 699. 2009. Tabii yapı taşları-Muayene ve deney metotları. Ankara: Turkish Standards Institution.

Uchida E, Ogawa Y, Maeda N, Nakagawa, T. 2000. Deterioration of stone materials in the Angkor monuments, Cambodia

EFFECT OF ROCK PROPERTIES ON WEATHERİNG PROCESSES, ŞAHMELİK TUFF ROCK MONUMENT

Mustafa KORKANÇ[1], İsmail İNCE[2], M. Ergün HATIR[3]

[1]Niğde Ömer Halisdemir University, Faculty of Engineering, Department of Geological Engineering, Niğde, Turkey
[2]Konya Technical University, Faculty of Engineering and Natural Sciences, Department of Geological Engineering, Selçuklu, Konya, Turkey
[3]Necmettin Erbakan University, Faculty of Fine Arts and Architecture, Department of Interior Architecture and Environmental Design, Meram, Konya, Turkey

Corresponding author e-mail: mkorkanc@ohu.edu.tr

ABSTRACT

Stone heritages are important monuments that transfer the cultural traces of past civilizations to the present day. These monuments are subject to deterioration over time as a result of atmospheric effects, threatening the integrity of cultural heritage. Monuments built with rocks with particularly weak strength properties are more sensitive to atmospheric processes. In this study, the deterioration developments and damages that occurred in the monument carved into the pyroclastic rock located in the Şahmelik neighborhood of Develi district, Kayseri province were examined. For this purpose, the physical-mechanical properties of the rock and the in-situ deterioration developments were examined in the laboratory using non-destructive testing techniques (Schmidt hardness, P-wave velocity and IR thermography). According to the findings obtained from the study, the rock has low density, high porosity and high capillary water absorption. These features increase the sensitivity of the rock from which the monument is carved to atmospheric deterioration processes. With these features, planning of urgent restoration-protection works will be very important in transferring the monument to future generations.

Keywords: Şahmelik Tuff Rock Monument, Deterioration, Non-destructive testing techniques, Atmospheric processes, Kayseri.

INTRODUCTION

Atmospheric weathering processes (such as freezing-thawing, wetting-drying and salt crystallization) primarily reduce the bonding strength of minerals on the surface of rocks and cause flaking type development on the mm scale (Hatır, 2020). In the continuation of these processes, different weathering types ranging from mm to m scale may develop. Especially in rocks with low strength and high porosity properties, these effects can cause destructive results more quickly. In addition to high porosity values, high capillary water absorption properties cause more water to be stored in the rock. Especially in winter, the expansion of the volume of water by freezing causes an increase in the existing pores in the rock (Fener and İnce, 2015).

As this process progresses, the rock loses integrity as a result of the increased pore volume. In the studies on monuments and historical structures from past to present, weathering has been stated to be directly related to atmospheric actions (İpekoğlu et al. 2007; Korkanç 2013; Brimblecombe 2014), precipitation affecting to the structure, groundwater (Sandrolini and Franzoni 2006; Fener and İnce 2015), petro-mechanics (Uchida et al. 2000) and hydraulics (Benavente et al. 2007) characteristics of the rock.

In this study, the deteriorations developing in Şahmelik Tuff Rock Monument, where the destructive effects of atmospheric deterioration processes were most intensely observed in the region, were investigated. The deterioration developments in this monument built during the Byzantine period were investigated by laboratory and on-site non-destructive tests.

MATERIALS AND METHOD

In this study, the rock unit in which the Şahmelik Tuff Rock Monument was carved was investigated by laboratory and on-site non-destructive test studies. For laboratory studies, uniform rock blocks with dimensions of 30x30x25 cm were collected from the field. Then, cube samples with dimensions of 7x7x7 cm were prepared from these blocks according to the principles specified in TS EN 1936 (2010). Using cube samples, dry density and porosity values of the rock were determined by taking into account the methods recommended in TS EN 1936 (2010). Capillary water absorption value of the samples was determined according to the methods recommended in TS EN 1925 (2000). In order to understand the deterioration processes by evaluating the weathered and fresh properties of the rock together, non-destructive tests (Schmidt hammer hardness, Leeb hardness and P-wave velocity) were applied both in the laboratory and on-site. From these tests, the P-wave velocity value was determined according to the method recommended in ASTM E494 (2010). In determining the Schmidt hammer hardness (SHR) value, the method specified in ASTM D5873 (2014) was taken into account. In determining the Leeb hardness, the method recommended in İnce and Bozdağ (2021) was used.

In the study, only the IR thermography method applied on-site was used with the FLIR brand E5 model device. The types of degradation observed in the monument were classified according to the principles defined in ICOMOS (2008).

RESULTS AND DISCUSSIONS

Physical-Strength Properties of the Rock

The physical-strength properties of the rock unit in which the Şahmelik Tuff Rock Monument was carved are 1.01 g/cm^3, 38.15% and 1.81 km/s, respectively. According to NBG (1985), this rock is in the very high porosity class, while its density is in the very low class. For example, the capillary water absorption value is 652.5 g/m^{2}s^{0,5} and according to Snethlage (2005), it is in the highly absorbing rock class. From the fresh surface hardness values of the rock, Schmidt hammer was determined as 22 while Leeb was determined as 208.

Deterioration types observed in the monument

Atmospheric deterioration processes can develop in a very small area in rocks on the mm scale, or they can develop on the m scale and cause loss of integrity in monuments. In Şahmelik Tuff Rock Monument, many types of weathering were detected from m to m scale. The beginning of weathering developments is related to the presence of water penetrating the monument as capillary. The high water absorption characteristics determined in fresh rock allow the rock body to penetrate water, causing atmospheric weathering processes to work effectively. Especially the presence of water in the capillary region caused the matrixes in the rock body to decrease their ability to adhere to each other, causing differential erosion type (Figure 1). In addition, as seen in Figure 1, missing part type weathering was detected in some weak structural elements on the differential erosion region.

Figure 1. Differential erosion and missing part type deteriorations observed in the Şahmelik Tuff Rock Monument

The development of the differential erosion type observed in the capillary region throughout the monument to depths of approximately 80 cm affected the static balance of the structure and triggered the formation of cracks in the interior spaces (walls and piers) and on the upper covers (Figure 2). As a result of the increase in the stress differences created by the cracks observed throughout the monument, large-scale block ruptures occurred (Figure 3). These ruptures caused the collapse of the upper cover and the collapse of the walls, leaving the monument even more vulnerable to atmospheric degradation processes.

Figure 2. Crack type deteriorations observed in the monument

Figure 3. Block breaks caused by cracks

Non-Destructive Test Results

Non-destructive test measurements were carried out on-site to determine the deterioration developments in the monument. In the surface hardness tests on fresh rock, Leeb and Schmidt hammer values were determined as 208 and 22, respectively. In the Leeb measurements made on-site, it was determined that it varied between 103-125 in the differential erosion zones and 136-165 above this zone. Schmidt hammer hardness values were determined as 10-12 and 14-16 in the differential erosion zone and above, respectively. In both hardness tests, a decrease of approximately 50% was observed in the differential erosion zones compared to the fresh rock values. While the P-wave speed test was determined as 1.81 km/s in the laboratory, no value was obtained in the differential erosion zone in the on-site analyses. The P-wave speed values above this zone were measured between 1.05 and 1.34 km/s. The visual of the IR thermography method applied to determine the humidity and temperature distributions in the structure is given in Figure 4. When Figure 4a is examined, differential erosion type deterioration is observed in the capillary region of the interior carrier column. In addition, crack type deterioration is observed diagonally cutting the column. When Figure 4b is examined, it is determined that the temperature is lower in relation to humidity along the differential erosion and crack zone.

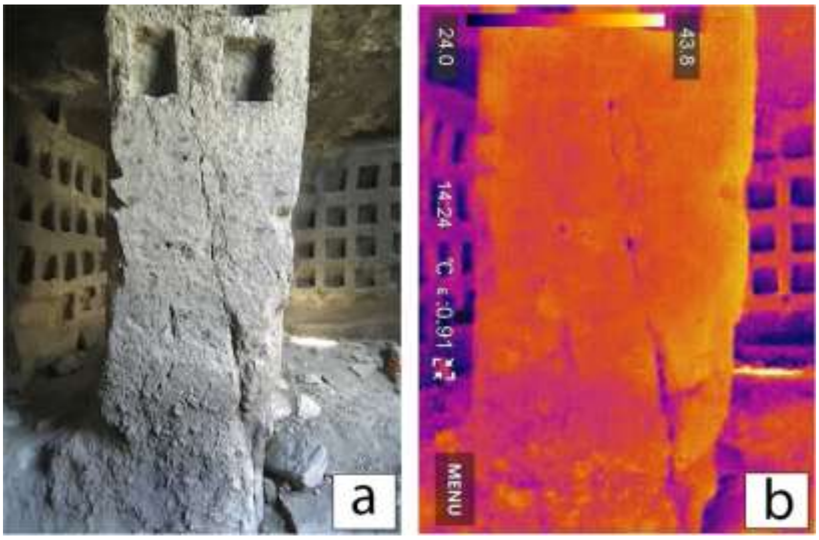


Figure 4. a) Interior image of the monument, b) IR thermography image

CONCLUSIONS

Rock properties play an important role in the deterioration of monuments built with stone. While rocks with high strength and low porosity are highly resistant to atmospheric effects, rocks with low engineering properties and high porosity are more sensitive to deterioration. In this study, deteriorations and structural problems in the Şahmelik Tuff Rock Monument, which was built on pyroclastic rock with low strength, high porosity and capillary water absorption properties, were examined.

- The structural integrity problems in the Şahmelik Tuff Rock Monument were triggered by differential erosion type deterioration developing in the capillary region due to the high water absorption properties of the rock.
- When the non-destructive tests applied in the laboratory and on-site were evaluated together, it was determined that the hardness values decreased by 25-50% compared to fresh rock. This decrease is the highest in the differential erosion region. In addition, the P-wave velocity value could not be obtained due to the excessive deterioration of this region.
- In the IR thermography images obtained from inside the structure, it was determined that the moisture content in the areas where differential erosion and crack type deterioration developed was also high.

REFERENCES

ASTM E494. 2010. Standard Practice for Measuring Ultrasonic Velocity in Materials. West Conshohocken: ASTM International.

Benavente D, Cueto N, Martínez-Martínez J, García del Cura MA, Cañaveras JC 2007. The influence of petrophysical properties on the salt weathering of porous building rocks, Environmental Geology, 52(2), 215-224.

Brimblecombe P. 2014. Environment and Architectural Stone, Stone in Architecture Properties, Durability eds. Siegesmund, S. and Snethlage, R., 317-348.

Fener, M., & İnce, İ. 2015. Effects of the freeze–thaw (F–T) cycle on the andesitic rocks (Sille-Konya/Turkey) used in construction building. Journal of African Earth Sciences, 109, 96-106.

Hatır, M. E. 2020. Determining the weathering classification of stone cultural heritage via the analytic hierarchy process and fuzzy inference system. Journal of Cultural Heritage, 44, 120-134.

İpekoğlu, B., Böke, H., Çizer, Ö., 2007. Assessment of material use in relation to climate in historical buildings, Building and Environment, 42: 970-978.

ICOMOS-ISCS. 2008. International Council on Monument and Sites International Scientific Committee for Stone, Illustrated Glossary on Stone Deterioration Patterns. Champigny/Marne, France: Ateliers 30 Impresión.

ISRM, 2007. The complete ISRM suggested methods for rock characterization, testing and monitoring: 1974–2006. In: Ulusay and Hudson (Eds.), Suggested methods prepared by the commission on testing methods, International Society for Rock Mechanics. ISRM Turkish National Group, Ankara, Turkey. 628 pp.

İnce, İ., & Bozdağ, A. 2021. An investigation on sample size in Leeb hardness test and prediction of some index properties of magmatic rocks. Arabian Journal of Geosciences, 14, 1-13.

Korkanç, M. 2013. Deterioration of different stones used in historical buildings within Nigde province, Cappadocia, Construction and Building Materials, 48: 789-803.

NBG. 1985. Engineering Geology and Rock Engineering, Handbook No. 2. 2nd ed. Trondheim, Norway: Norwegian Group for Rock Mechanics (NBG).

Sandrolini, F, Franzoni, E. 2006. An operative protocol for reliable measurements of moisture in porous materials of ancient buildings, Building and Environment, 41: 1372-1380.

Snethlage, R., 2005. Leitfaden Steinkonservierung. Fraunhofer IRB, Stuttgart

TS EN 1925 2000. Natural stone test methods determination of water absorption coefficient by capillarity. Turkish Standards Institution, Ankara

TS EN-1936. 2010. Natural Stone Test Methods – Determination of Real Density and Apparent Density and of Total and Open Porosity. Ankara: Turkish Standards Institution.

Uchida E, Ogawa Y, Maeda N, Nakagawa, T. 2000. Deterioration of stone materials in the Angkor monuments, Cambodia, Engineering Geology, 55(1-2): 101-112.

USING SPI AND RDI TO ASSESS THE IMPACT OF CLIMATE CHANGE ON METEOROLOGICAL DROUGHT: A CASE STUDY OF EDIRNE, TÜRKIYE

Zinnur YILMAZ[1], Mustafa Bünyamin KARAGÖZOĞLU[1]

[1] Sivas Cumhuriyet University, Engineering Faculty, Department of Environmental Engineering, Sivas, Turkiye
Corresponding author e-mail: zinnuryilmaz@cumhuriyet.edu.tr

ABSTRACT

Drought is a natural phenomenon characterized by a significant decrease in precipitation levels in a given region. This condition can lead to the desiccation of soil and water sources, as well as damage to vegetation and a decline in agricultural productivity. Droughts can arise from the interaction of various factors and typically have serious implications for ecosystems. Consequences may include water scarcity, food security issues, and economic losses. Consequently, drought represents a significant concern from both environmental and socio-economic perspectives. Global warming and climate change have led to more frequent and severe droughts worldwide, including in Turkey. Water resources and the agricultural sector are the most affected by droughts. In this study, drought analysis was conducted in Edirne province, located in the Thrace region of Turkey. Annual average rainfall, maximum temperature, minimum temperature, and average temperature data were used for analysis. Forty years of rainfall data (1984-2023) were analyzed. The Standardized Precipitation Index (SPI) and Reconnaissance Drought Index (RDI) methods were used for drought analysis. SPI and RDI values were calculated for 1-, 3-, 6-, and 12-month periods, and the severity, duration, and distribution of drought periods were determined separately for each station. Overall, examining the values calculated with SPI and RDI, it was observed that the years 1985, 1986, 1989, and 2013 were generally drought in the study area.
Keywords: climate, climate change, drought analysis, Edirne, RDI, SPI

1. INTRODUCTION

Climate change stands as one of the most pressing environmental challenges of our time, with its effects extending beyond mere temperature increases to significant alterations in precipitation patterns, thereby increasing the frequency and intensity of drought events. Drought, as one of the most prominent manifestations of climate change, is a complex phenomenon that needs to be addressed in both meteorological and hydrological contexts. Meteorological drought, initiated by a decline in precipitation, gradually evolves into hydrological drought, leading to reduced water resources and ecosystem degradation (Soğanci 2019; Kale 2021; Erişmiş 2023). Consequently, drought poses serious threats across various sectors, from agricultural production to water resources, endangering food security (Morán-Tejéda et al. 2012; Sharafati et al. 2019).

Drought analysis emerges as a crucial tool in understanding and managing the impacts of climate change. Methods such as the Standardized Precipitation Index (SPI), Standardized Precipitation Evapotranspiration Index (SPEI), and Reconnaissance Drought Index (RDI) are commonly used to evaluate drought conditions in specific regions. SPI measures deviations of precipitation over a given period relative to the normal distribution, while SPEI considers the balance between precipitation and evaporation in assessing drought conditions (Mohammed et al. 2020; Chisadza et al. 2023). Research has demonstrated the effectiveness of SPI and SPEI in assessing drought conditions and highlighting the adverse impacts of climate change. For instance, long-term drought analyses in the Meriç River Basin have detailed the impacts of climate change in the region (Erişmiş 2023), while studies in the Akarçay Closed Basin have illustrated the effects of temperature and precipitation anomalies on the hydrological cycle (Kale 2021). These analyses not only clarify the impacts of drought on agricultural production but also help understand farmers' responses to drought (Çuhadar and Atiş 2021; Kara and Yereli 2022). Additionally, these indices provide a comprehensive understanding of drought persistence and intensity, aiding in the development of necessary strategies for water resource management (Stagge et al. 2015).

Drought analysis goes beyond evaluating meteorological data, offering a broader perspective by incorporating future climate scenarios. For example, the work of Stagge et al. examines how indices like SPI, SPEI, and RDI change under climate change scenarios, underscoring their importance in assessing drought conditions (Stagge et al. 2015; Sharafi and Ghaleni 2022). Such studies are pivotal for identifying future drought scenarios and developing effective water management strategies.

Drought is not merely a natural disaster but also a socio-economic challenge. Losses in agricultural production threaten food security and may trigger migration in rural areas (Beden et al. 2020; Ekinci and Ökde 2021). In Turkey, developing strategies to combat climate change is of paramount importance in addressing these issues. The role of local governments is critical in this process, being key elements in combating climate change (Beden et al. 2020; Ekinci and Ökde 2021). In Turkey, developing strategies to combat climate change is of paramount importance in addressing these issues. The role of local governments is critical in this process, being key elements in combating climate change (Parlak 2022; Uysal 2022). Understanding the relationship between drought and climate change is essential for developing sustainable agricultural policies and water management strategies (Türkeş 2012).

In conclusion, climate change and drought analysis are complex issues that need to be addressed with their environmental and social dimensions. The use of indices such as SPI and RDI is crucial for evaluating drought conditions and mitigating their effects. Therefore, adopting a multidisciplinary approach in combating climate change is a critical necessity from both scientific and societal perspectives. Drought analysis plays a significant role in evaluating future climate scenarios and implementing effective measures.

Located in northwestern Turkey, Edirne is a critical region for agriculture and water resources management. Understanding the impacts of climate change in this region is vital for developing sustainable agriculture and water management strategies. In this context, meteorological drought indicators such as the Standard Precipitation Index (SPI) and the Reconnaissance Drought Index (RDI) are important tools for analyzing drought events in the region. This study aims to evaluate the impacts of climate change on meteorological drought in the Edirne region through SPI and RDI analyses. The findings will shed light on water management and agricultural planning strategies in the region and contribute to the development of measures to mitigate future drought risks. This study will be an important step towards achieving sustainable development goals in Edirne and similar regions.

2. METHOD

2.1. Study Area and Climate Data

Edirne, located in the northwestern part of Turkey, is a province rich in historical heritage. Geographically, Edirne is situated along the banks of the Meriç River, which serves as a significant water source for the region. This proximity to the river directly influences the area's climate and meteorological parameters. Edirne typically experiences a continental climate characterized by hot and dry summers, and cold and wet winters (Oruc 2021).

Covering approximately 1% of Turkey's total land area, Edirne's catchment area spans 6,279 km². The province is under the influence of a transitional climate between the continental and Mediterranean climates. The average annual precipitation in Edirne is 589.1 mm/m², while the annual average temperature is 13.7°C. These climatic characteristics play a crucial role in shaping Edirne's water resources and agricultural activities.
For the drought analysis of Edirne, meteorological data was sourced from the NASA POWER website. The analysis utilized observed data on annual average total precipitation, annual maximum temperature, annual minimum temperature, and annual average temperature. General information about the study area is provided in Table 1 and Figure 2.

Table 1. General Information About Meteorological Stations

Province	Latitude	Longitude	Altitude (km)	Year	P_{mean} (mm)	T_{min} (°C)	T_{max} (°C)	T_{mean} (°C)
Edirne	36.7411	35.143	0.39	1984 - 2023	563.765	7.826	19.617	13.336

Figure 1. Location Map of Edirne Province

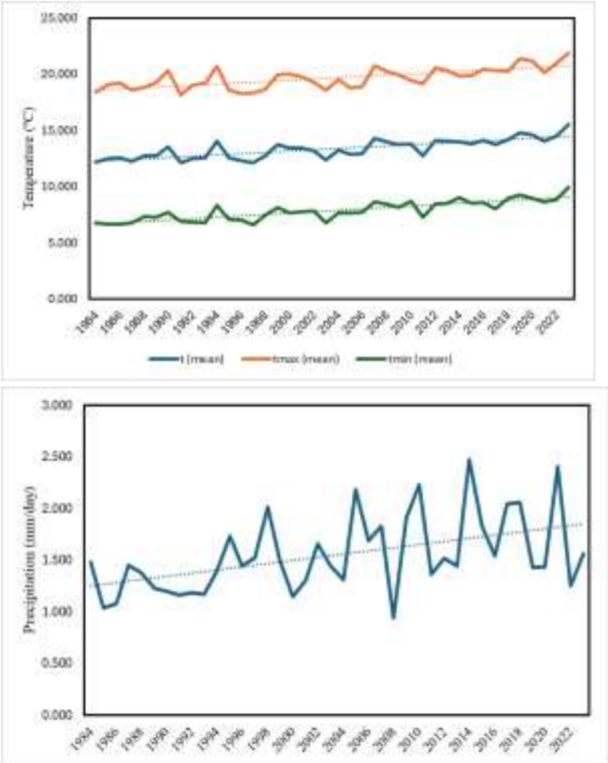


Figure 2. Temperature and Precipitation Graphs of Edirne Province by Years

2.2. Standard Precipitation Index (SPI)

The Standardized Precipitation Index (SPI) is an important tool used to assess meteorological droughts. Proposed by McKee and colleagues in 1993, this index measures the deviation of precipitation amounts from the normal distribution over a specific time period (typically 1, 3, 6, 12, or 24 months). SPI has been recognized by the World Meteorological Organization (WMO) as a reference index for drought monitoring and climate risk management. (Vicente-Serrano et al. 2012; Hao and AghaKouchak 2013). This index is obtained by dividing the difference between precipitation in a specific time period and the average precipitation by the standard deviation:

$$SPI_{i,j} = \frac{X_{i,j} - X_j^m}{\sigma_j} \qquad (1)$$

In Equation 1, $X_{i,j}$, represents the precipitation (mm) in month j of year i, X_j is the average precipitation (mm) for month j, and σ_j is the standard deviation of precipitation for month j. The SPI can be calculated for different periods (such as 1, 3, 6, 9, or 12 months).

The SPI (Standardized Precipitation Index) method assesses drought severity by calculating precipitation deficits for different time series during both wet and dry periods. Positive values calculated by this method indicate wet periods, while negative values represent dry periods. When calculating SPI, long-term precipitation data are fitted to a gamma distribution, and these values are then transformed into a normal distribution (Edwards and McKee 1997). This approach allows for consistent representation across different climatic conditions.

In drought assessment, a period where the index remains continuously negative is defined as a drought period. The month when the index first falls below zero is considered the onset of drought, and the month when it rises to positive values is regarded as the end of the drought (McKee et al. 1995).

2.3. Reconnaissance Drought Index (RDI)

The Reconnaissance Drought Index (RDI) is a tool developed for assessing meteorological droughts and is considered an effective instrument for analyzing drought conditions, especially in arid and semi-arid regions. RDI takes into account the water balance by using precipitation and potential evapotranspiration (PET) data, thus providing a more comprehensive evaluation of drought conditions (Waseem et al. 2016; Yisehak et al. 2021).

$$\alpha_k^i = \frac{\sum_{j=1}^{k} Pij}{\sum_{j=1}^{k} PETij}, i = 1\,(1)\,N and j = 1\,(1)\,K$$

P_{ij} and PET_{ij} represent the cumulative precipitation and potential evapotranspiration for the j-th month of the i-th year, respectively, and n is the total number of years of the available data.

$$RDI^i = \frac{Y^i - \bar{Y}}{\hat{\delta}_y}$$

Y^i represents the arithmetic mean, and Y denotes the standard deviation. In the case of applying the gamma distribution, the RDI can be calculated by fitting the gamma probability density function (PDF) to the given frequency distribution (Tigkas 2008; Tsakiris et al. 2008). For cumulative precipitation periods that may include zero values, such as short reference periods (e.g., monthly or 3-month), the RDI can be computed according to a composite cumulative distribution function that incorporates the probability of zero precipitation and the gamma cumulative probability

Table 2. Classification of Drought Conditions According to SPI and RDI

SPI or RDI Values	SPI or RDI Classification
X>2.00	Extremely Wet
1,99 – 1,50	Very Wet
1,49 – 1,00	Moderately Wet
0,99 – (-0,99)	Near Normal
(-1,00) – (-1,49)	Moderately Dry
(-1,50) – (-1,99)	Severely Dry
-2,00 ≤ X	Extremely Dry

3. RESULTS

3.1. Drought Analysis of Edirne

3.1.1. Drought analysis based on SPI

The temporal distribution of SPI values for 1-, 3-, 6-, and 12-month periods, calculated using monthly average precipitation data for Edirne from 1984 to 2023, is shown in Figure 3. According to Figure 3, the frequency of drought periods increases with the length of the period. The analysis reveals that the highest droughts for the 1-, 3-, 6-, and 12-month periods were observed in the years 2015, 2002, 1986, and 1985, respectively. The wettest periods occurred in the years 2014, 2021, 2010, and 2005, respectively.

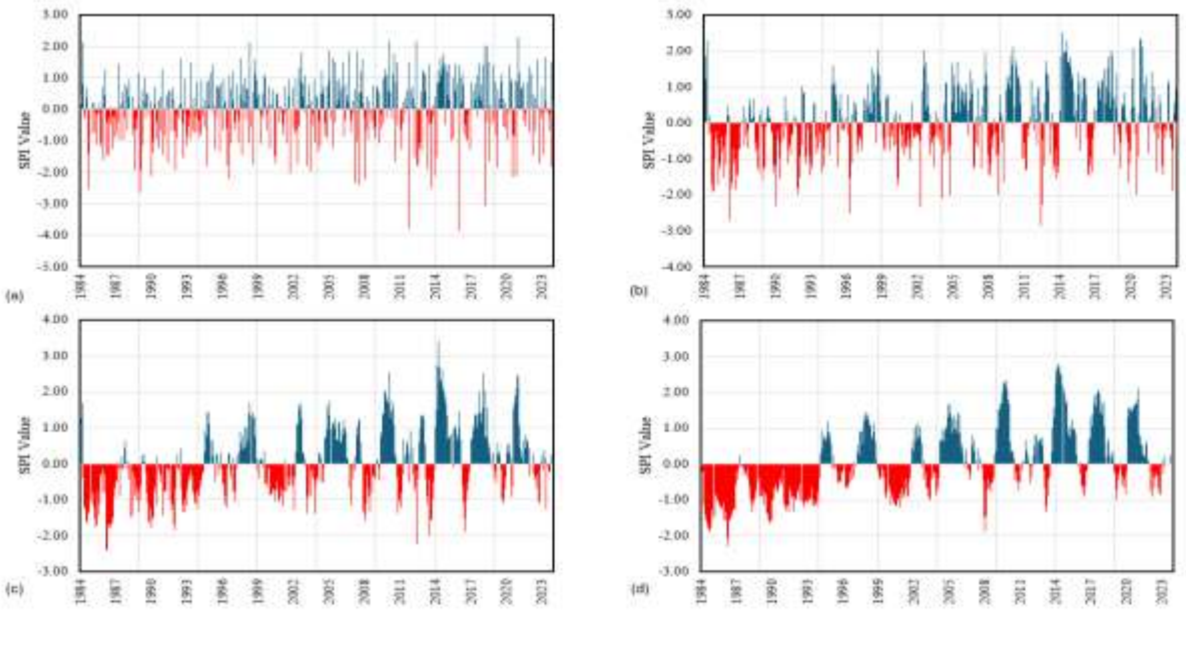


Figure 3. Temporal distribution of Edirne station SPI (a)SPI1 b)SPI3 c)SPI6 d)SPI12) values.

The SPI analyses revealed long-term drought periods over different time intervals. According to the SPI1 analysis, a 7-month drought period occurred from June 2016 to December 2016. The SPI3 analysis showed a drought lasting a total of 19 months from July 1984 to January 1986. The SPI6 analysis, which provides a longer-term assessment, identified a 40-month drought period from April 1984 to July 1987. The longest drought, as indicated by the SPI12 analysis, persisted for 92 months from May 1987 to December 1994. These data indicate that long-term droughts occurred at different intervals in the Edirne region, with drought conditions persisting significantly (Table 3). Additionally, it is observed that as the periods for SPI extend, there is an increase in the number of months affected by drought.

Table 3. Duration, severity and maximum occurrence values (SPI) of drought at Burdur station

.

Period	Time (Month)	Start	Finish	Pick Value	Pick Time
SPI1	7	Jun.16	Dec.16	-3.88	Dec.15
SPI3	19	Jul.84	Jan.86	-2.87	Jun-Aug.12
SPI6	40	Apr.84	Jul.87	-2.42	Mar-Aug.86
SPI12	92	May.87	Dec.94	-2.30	Mar.86-Feb.87

Figure 4 shows the percentage distribution of drought categories for SPI1, SPI3, SPI6, and SPI12 periods. The drought categories are classified as EW (Extremely Wet), SW (Severely Wet), MOW (Moderately Wet), MW (Mild Wet), MD (Mild Drought), MOD (Moderate Drought), SD (Severe Drought), and ED (Extreme Drought). In the SPI1 graph, the highest percentage is represented by the Mild Wet (MW) category at 39.2%, indicating that mild wet periods are more frequent in the short-term period. This is followed by the Mild Drought (MD) category at 30.2%. Extremely Wet (EW) and Extreme Drought (ED) periods have the lowest percentages, 1.3% and 3.1% respectively, suggesting that extreme conditions are less common during the SPI1 period.

In SPI3, the Mild Drought (MD) category stands out as the most common condition at 34.6%, followed by Mild Wet (MW) at 30.8%. Moderately Wet (MOW) and Moderate Drought (MOD) categories are observed at similar levels, 10.8% and 10.0%, respectively, while Extremely Wet (EW) and Extreme Drought (ED) periods are relatively lower at 2.3% and 1.9%.

For the SPI6 period, Mild Drought (MD) is the most prevalent condition at 38.1%, followed by Mild Wet (MW) at 27.9%. This suggests that drought conditions become more pronounced in longer periods. Moderate Drought (MOD) is third at

11.0%, while Extreme Drought (ED) is quite rare at 0.6%. Extremely Wet (EW) conditions are at 3.3% for this period.

In SPI12, Mild Drought (MD) has the highest percentage at 41.1%, indicating that long-term droughts significantly impact the region. Mild Wet (MW) is second at 26.7%. Extreme Drought (ED) is very low at 0.4%, and Extremely Wet (EW) is also low at 3.8%, suggesting that extreme conditions are rare over long periods.

Overall, SPI analyses show that drought conditions become more pronounced as the time period increases (from SPI1 to SPI12) and drought periods become more common. Mild Drought (MD) is the dominant category across all SPI periods, indicating that the region generally experiences mild drought conditions. The proportions of Extreme Drought and Extremely Wet conditions remain low, especially in longer periods, indicating that extreme conditions are less frequent. These data provide valuable insights into the general climatic dynamics of the Edirne region and are crucial for developing water resource management strategies.

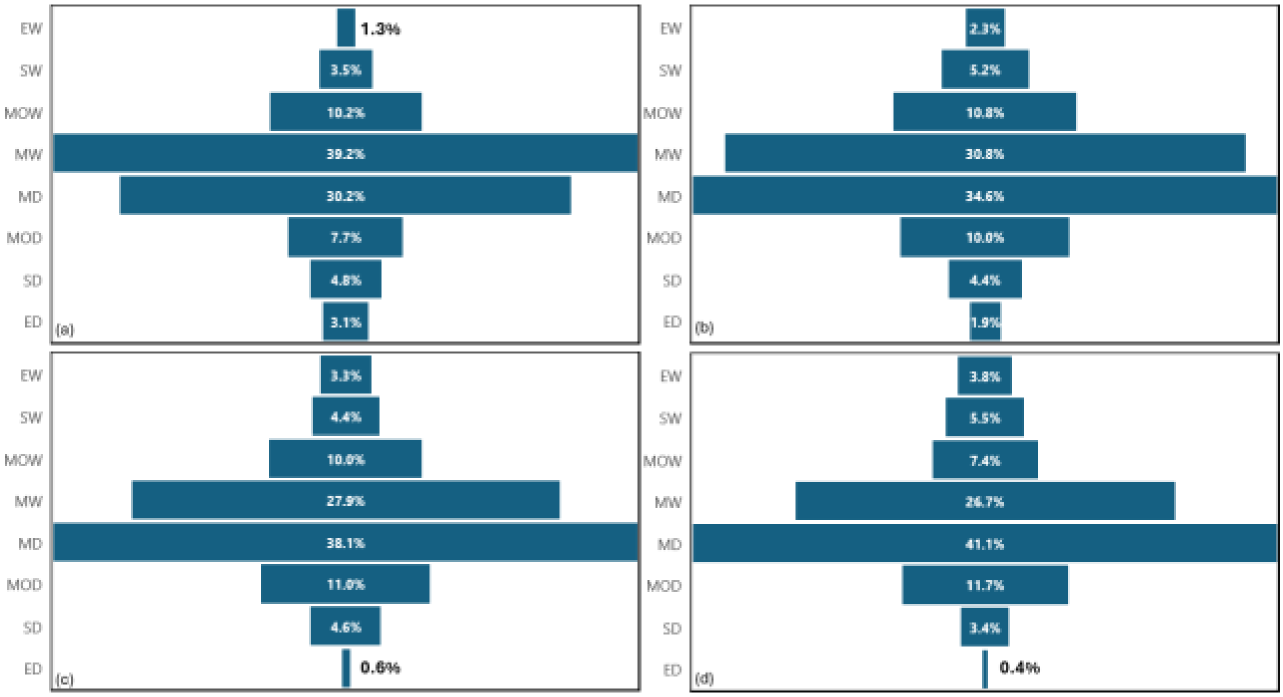


Figure 4. Occurrence percentages of drought classes (SPI) at Edirne station.

3.1.2. Drought analysis based on RDI

The temporal distribution of RDI values for 1-, 3-, 6-, and 12-month periods, calculated using monthly average precipitation data for Edirne from 1984 to 2023, is shown in Figure 5. According to Figure 5, the frequency of drought periods increases with the length of the period. The analysis reveals that the highest droughts for the 1-, 3-, 6-, and 12-month periods occurred in the years 1990, 1986, 1986, and 1986, respectively. The wettest period were experienced in the year 2014.

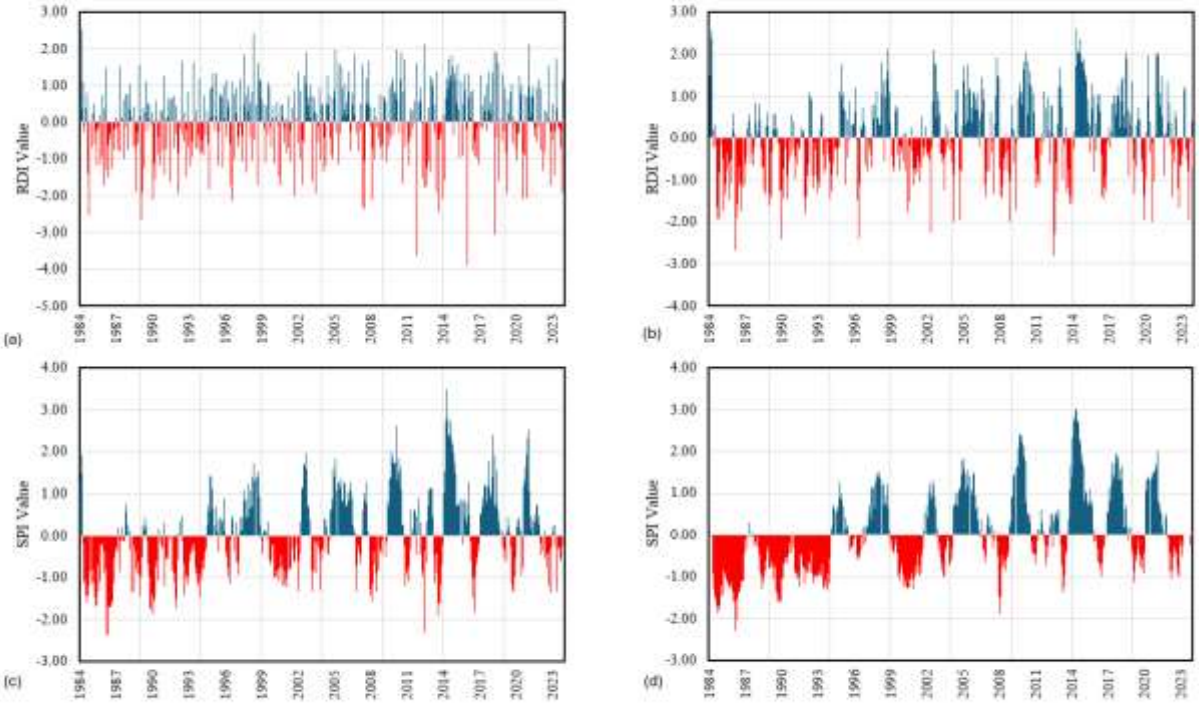


Figure 5. Temporal distribution of Edirne station RDI (a)RDI1 b)RDI3 c)RDI6 d)RDI12) values.

The RDI analyses clearly reveal the presence of long-term drought periods in the Edirne region over different time intervals (Table 4). According to the RDI1 analysis, a 7-month drought period occurred from June 2016 to December 2016. This indicates a short-term drought scenario, suggesting that seasonal effects triggered the drought conditions. The RDI3 analysis, on the other hand, points to a 15-month drought period from September 1989 to November 1990. This period extends beyond seasonal drought effects and indicates significant pressure on water resources on an annual basis.

Looking at the longer time frame of the RDI6 analysis, a 40-month drought period was identified from April 1984 to July 1987. This extended drought period could have serious impacts on both hydrological and agricultural activities, highlighting the importance of water management strategies. The longest drought, as indicated by the RDI12 analysis, encompasses a 51-month period from August 1987 to October 1990. This suggests that long-term droughts are also a significant threat in the region and underscores the necessity for long-term planning in water resource management.

Overall, RDI analyses show that as the periods extend, the number of months experiencing drought also increases. This situation indicates that drought events can span significantly longer periods rather than being short-term, necessitating comprehensive measures and strategies. These analyses provide a crucial foundation for measures against climate change and drought risks in the Edirne region.

Table 4. Duration, severity and maximum occurrence values (SPI) of drought at Edirne station.

Period	Time (Month)	Start	Finish	Pick Value	Pick Time
RDI1	7	Jun.16	Dec.16	-3.90	Dec.15
RDI3	15	Sep.89	Nov.90	-2.82	Jun-Aug.12
RDI6	40	Apr.84	Jul.87	-2.39	Apr-Sep.86
RDI12	51	Aug.87	Oct.90	-2.28	Mar.86-Feb.87

According to the RDI analyses, the distribution of drought conditions in the Edirne region varies significantly across different periods. In the RDI1 analysis, the most common drought categories are Medium Wet (MW) at 36.5% and Mild Drought (MD) at 31.9%. As the time period extends, there is a notable increase in the proportion of the Mild Drought (MD) category, which rises to 40.6% in the RDI12 analysis. This indicates that, particularly in long-term analyses, drought conditions become more moderately prevalent, with serious drought risks increasing over time (Figure 6).

On the other hand, more extreme drought categories such as Extreme Drought (ED) and Severe Drought (SD) remain at low percentages across all periods. For instance, in the RDI12 analysis, the percentage of Extreme Drought (ED) is only 0.4%, suggesting that extreme drought events are rare. The dominance of the Medium and Mild Drought categories indicates that drought risks in the region are more often characterized by long-term but less severe droughts. These findings are critical for the development of long-term water management and agricultural planning strategies in the Edirne region. The increasing prevalence of moderate drought conditions heightens the pressure on the region's water resources and underscores the importance of implementing effective drought management measures.

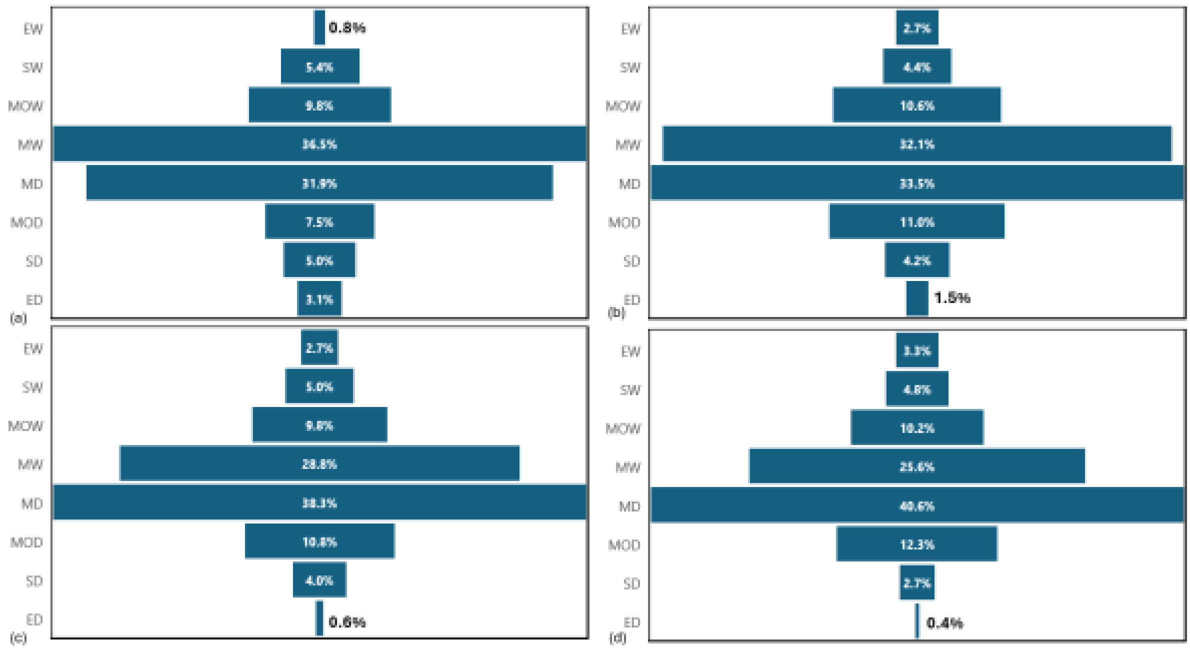


Figure 6. Occurrence percentages of drought classes (RDI) at Edirne station.

4. CONCLUSION

This study conducted meteorological and agricultural drought analyses for Edirne central district, located in the Thrace Region. To achieve this, average precipitation, and average minimum and maximum temperatures observed in Edirne from 1984 to 2023 were used to analyze meteorological and agricultural droughts. By applying the Standardized Precipitation Index (SPI) and the Reconnaissance Drought Index (RDI) methods to the data, the severity, magnitude, and distribution of dry and wet periods were determined for SPI and RDI values calculated for 1, 3, 6, and 12-month periods.

Upon examining the SPI results, it was observed that there were 7, 19, 40, and 92 dry months in Edirne during 1, 3, 6, and 12-month periods between 1984 and 2023, respectively. During the studied period, the maximum droughts in Edirne station for 1, 3, 6, and 12-month periods occurred in December 2015, June-August 2012, March-August 1986, and March 1986-February 1987, respectively.

Upon examining the RDI results, it was observed that there were 7, 15, 40, and 51 dry months in Edirne during 1, 3, 6, and 12-month periods between 1984 and 2023, respectively. During the studied period, the maximum droughts in the Edirne station for 1, 3, 6, and 12-month periods occurred in December 2015, June-August 2012, April-September 1986, and March 1986-February 1987, respectively.

Overall, examining the values calculated with SPI and RDI, it was observed that the years 1985, 1986, 1989, and 2013 were generally dry in the study area. The analyses indicated that the severity and duration of droughts have increased in recent years in the Edirne Basin, and agricultural droughts have also been observed alongside meteorological droughts.

To combat the devastating effects of drought in the Thrace Basin, an area significant for agricultural resources in our country, it would be beneficial to regularly monitor the variability in climate data and develop local policies that ensure the effective management of water resources. Additionally, identifying hydrological droughts and precipitation trends for the same region would provide a valuable resource for water management studies.

REFERENCES

Beden N, Demir V, Keski̇n AÜ. 2020. Samsun İlinde SPI ve PNI Kuraklık İndekslerinin Eğilim Analizi. Deu Muhendislik Fakultesi Fen Ve Muhendislik 22:107–116. https://doi.org/10.21205/deufmd.2020226411

Chisadza B, Gwate O, Ncube F, Mpofu N. 2023. Assessment and characterisation of hydrometeorological droughts in the Upper Mzingwane sub-catchment of Zimbabwe. Natural Hazards 116:3275–3299. https://doi.org/10.1007/s11069-022-05807-9

Çuhadar M, Atiş E. 2021. Üreticilerin Kuraklığa Yönelik Tutumlarını Etkileyen Temel Faktörlerin Analizi. Türk Tarım Ve Doğa Bilimleri Dergisi 8:1–7. https://doi.org/10.30910/turkjans.765182

Edwards DC, McKee TB. 1997. Characteristics of 20th century drought in the United States at multiple time scales. Department of Atmospheric Science, Colorado State University, Fort Collins, Colorado

Ekinci E, Ökde F. 2021. İklim Değişikliğinin Kırsal Alanlara Etkisinin Muhtarlar Üzerinden Değerlendirilmesi: Yozgat Köyleri Örneğinde. Afet Ve Risk Dergisi 4:300–318. https://doi.org/10.35341/afet.993555

Eri̇şmi̇ş M. 2023. Standartlaştırılmış yağış buharlaşma indeksine göre Meriç Nehri Havzasında uzun dönem kuraklık analizi. Lnternational Journal of Geography and Geography Education 313–328. https://doi.org/10.32003/igge.1297107

Hao Z, AghaKouchak A. 2013. Multivariate Standardized Drought Index: A parametric multi-index model. Advances in Water Resources 57:12–18. https://doi.org/10.1016/j.advwatres.2013.03.009

Kale MM. 2021. Akarçay Kapalı Havzası için Hidrolojik Kuraklık Analizi. Journal of Geography 165–180. https://doi.org/10.26650/jgeog2021-892360

Kara KÖ, Yereli AB. 2022. İklim Değişikliğinin Yönetimi ve Tarım Sektörü. Afet Ve Risk Dergisi 5:361–379. https://doi.org/10.35341/afet.1100932

McKee TB, Doesken NJ, Kleist J. 1995. Drought monitoring with multiple time scales. american meteorological society. Dallas, pp 233–236

Mohammed S, Alsafadi K, Daher HO, et al. 2020. Precipitation pattern changes and response of vegetation to drought variability in the eastern Hungary. Bulletin of the National Research Centre 44:. https://doi.org/10.1186/s42269-020-00306-y

Morán-Tejéda E, Ceglar A, Medved-Cvikl B, et al. 2012. Assessing the capability of multi-scale drought datasets to quantify drought severity and to identify drought impacts: an example in the Ebro Basin. International Journal of Climatology 33:1884–1897. https://doi.org/10.1002/joc.3555

Oruc S. 2021. Climate Extreme Indices Derived from Observed Daily Temperature Data Over Three Cities of Thrace Region, Turkey. In: Proceedings Article. Alanya Üniversitesi, Alanya,

Parlak C. 2022 The Role of Local Governments to Combat Climate Change: A Research Through the Authorization and Responsibilities. Resilience 6:321–334. https://doi.org/10.32569/resilience.1210273

Sharafati A, Nabaei S, Shahid S. 2019. Spatial assessment of meteorological drought features over different climate regions in Iran. International Journal of Climatology 40:1884–1884. https://doi.org/10.1002/joc.6307

Sharafi S, Ghaleni MM. 2022. Spatial assessment of drought features over different climates and seasons across Iran. Theor Appl Climatol 147:941–957. https://doi.org/10.1007/s00704-021-03853-0

Soğanci S. 2019. Küresel İklim Değişikliğinin Kaçınılmaz Sonucu: Kuraklık. Resilience 3:. https://doi.org/10.32569/resilience.619219

Stagge JH, Tallaksen LM, Gudmundsson L, et al. 2015. Candidate Distributions for Climatological Drought Indices (<scp>SPI</scp> and <scp>SPEI</scp>). International Journal of Climatology 35:4027–4040. https://doi.org/10.1002/joc.4267

Türkeş M. 2012. Türkiye’de gözlenen ve öngörülen iklim değişikliği, kuraklık ve çölleşme. Ankara Üniversitesi Çevrebilimleri Dergisi 4:1–32. https://doi.org/10.1501/csaum_0000000063

Uysal Y. 2022. İklim Değişikliği ve Küresel Isınma ile Mücadelede Yerel Yönetimlerin Rolü: Tespitler ve Öneriler. Kesit Akademi 30:324–354. https://doi.org/10.29228/kesit.57382

Vicente-Serrano SM, Beguería S, Lorenzo-Lacruz J, et al. 2012. Performance of Drought Indices for Ecological, Agricultural, and Hydrological Applications. Earth Interactions 16:1–27. https://doi.org/10.1175/2012ei000434.1

Waseem M, Ajmal M, Lee JH, Kim T-W. 2016. Multivariate Drought Assessment Considering the Antecedent Drought Conditions. Water Resources Management 30:. https://doi.org/10.1007/s11269-016-1416-5

Yisehak B, Shiferaw H, Abrha H, et al. 2021. Spatio-temporal characteristics of meteorological drought under changing climate in semi-arid region of northern Ethiopia. Environmental Systems Research 10:21. https://doi.org/10.1186/s40068-021-00226-4

MONITORING SPATIAL AND COASTAL LINE CHANGES IN THE SIVAS 4 EYLÜL DAM USING SATELLITE IMAGERY

Zinnur YILMAZ[1], Mustafa Bünyamin KARAGÖZOĞLU[1]

[1] Sivas Cumhuriyet University, Engineering Faculty, Department of Environmental Engineering, Sivas, Turkiye
Corresponding author e-mail: zinnuryilmaz@cumhuriyet.edu.tr

ABSTRACT

The effects of global warming not only alter hydrological systems but also exacerbate environmental issues such as drought and depletion of water resources. This situation increases pressure on water sources and causes harm to ecosystems, including water scarcity, erosion, and habitat loss. Particularly, the increase in frequency of extreme weather events further complicates the efficiency and sustainability of existing water resources. Therefore, water management and infrastructure planning require more sophisticated and knowledge-based approaches. Technologies such as remote sensing (RS), Geographic Information Systems (GIS), and Machine Learning (ML) can assist in making more efficient and robust decisions regarding the management of water resources and the selection of dam sites. These technologies can help optimize the utilization of current and future water resources and minimize environmental impacts. This, in turn, supports the sustainability of water resources and the development of more effective strategies to meet the water needs of communities. Consequently, advancements in satellite technology and computational power have expanded the capacity to manage various hydrological parameters and land characteristics. Conducting hydrological analyses through the integration of remote sensing and geographic information systems offers significant benefits in terms of both cost and time efficiency. In this study, according to DSI data, Sivas 4 September Dam, which decreased to 0 %in 2022, was examined in volumetric and spatial changes. Additionally, the correlation between annual temperature and precipitation levels and their impact on the water surface area of the dam was examined to identify the relationship between these variables.

Keywords: climate, climate change, global warming, monitoring, Satellite Imagery, Sivas

1. INTRODUCTION

Climate change is currently recognized as one of the most critical global challenges in terms of environmental sustainability and human health. The impacts of these changes are observed in various areas, such as rising temperatures, alterations in precipitation patterns, sea level rise, and the degradation of ecosystems. While reservoirs play essential roles in functions such as water management, energy production, and agricultural irrigation, increasing temperatures, shifting precipitation patterns, and heightened evaporation due to climate change negatively affect water levels in these reservoirs (Zhao and Gao 2018; Hou et al. 2022). Therefore, monitoring and assessing the effects of climate change have become more systematic and efficient through the use of modern technologies such as remote sensing and geographic information systems (GIS). Remote sensing, using satellite data, plays a crucial role in monitoring various parameters such as temperature variations, precipitation regimes, and changes in vegetation cover (Özdemir, 2023; Uslu et al., 2021; Softaoğlu, 2023; Roman et al., 2016; Binarti & Santoso, 2022).

Although freshwater lakes make up only 0.007% of the Earth's total water reserves, they account for 2.53% of the total freshwater reserves and are a crucial part of the water cycle (Shiklomanov, 1993). Lakes and reservoirs are the most accessible and usable water sources for human consumption and ecosystems. For this reason, observation stations have been established on lakes and reservoirs in many regions worldwide. However, field measurements require significant effort, time, and high costs, making it difficult to monitor many water sources in remote areas (Rahmani, 2021). Furthermore, while observation stations generally measure only water levels, the area and volume of water resources remain unmeasured. Monitoring both the surface area and volume of water resources is essential for understanding their responses to climate change on both regional and global scales (Jha et al., 2006). In this context, advancements in remote sensing and Geographic Information Systems (GIS) technologies have provided scientists with innovative approaches to monitor the surface areas and levels of water bodies (Cappelaere et al., 2003).

Numerous methods have been developed for the detection and extraction of water bodies from satellite imagery. One of the earliest studies in this field was conducted by Dolan et al. (1991), which examined the potential of satellite imagery in detecting water bodies. Gao (1996) emphasized the role of spectral bands in identifying water surfaces and demonstrated that these bands can lead to more accurate water body detection. The Normalized Difference Water Index (NDWI), developed by McFeeters (1996), has become a widely used method for water body identification. Braud and Feng (1998) explored the applicability of remote sensing techniques for monitoring water surfaces and noted that these techniques can play a significant role in water resource management. Frazier and Page (2000) compared different algorithms used for water body detection and identified the most effective methods. Xu (2006) demonstrated how satellite images can be utilized to calculate the surface areas of water bodies. Shen and Li (2010) examined the integration of remote sensing data in monitoring water bodies and provided recommendations on how this data can be used for water management. Feyisa et al. (2014) evaluated the effectiveness of various indices used to identify water surfaces and highlighted their importance as tools for monitoring water bodies. Water reflection has a lower value in near-infrared bands compared to land areas and is almost close to zero. This characteristic makes water bodies appear darker in satellite images, facilitating easier extraction (Raju et al., 2015). Shih (1985) demonstrated that the combination of bands from Landsat and other satellite sensors can be used to classify lake water surfaces. In this context, monitoring the surface areas of water resources using satellite images is of great importance for water management and environmental monitoring.

Remote sensing and GIS are also effectively utilized for monitoring and detecting changes related to climate change. Bhatt (2015) explained how these technologies can be used to determine the status of objects or phenomena across different time periods. Cuca (2017) emphasized the use of remote sensing to monitor the impacts of climate change on cultural and natural heritage, highlighting the importance of this data in the development of management strategies.
Climate change has significant impacts on water resources and dams. While dams play critical roles in water management, energy production, and agricultural irrigation, the rising temperatures, altered precipitation patterns, and increased evaporation rates resulting from climate change directly affect the water levels and functionality of dams (Null et al., 2013; Biglarbeigi et al., 2020). Marcé and colleagues examined the effects of climate change on water temperatures and emphasized the negative impacts these changes have on the ecological balance of dams (Marcé et al., 2010). Null and colleagues discussed how dams influence water temperatures and explored their potential to mitigate the effects of climate change (Null et al., 2013). Biglarbeigi and colleagues developed a model to assess the performance of dams under climate change scenarios, addressing changes in water levels and the challenges in water management, thereby contributing to the development of adaptation strategies (Biglarbeigi et al., 2020). Granados and colleagues analyzed the role of dam storage capacity in response to climate change and highlighted its impact on the reliability of water supply systems (Granados et al., 2021). Uysal and Doğan (2022) evaluated the effects of climate change on dam management and emphasized the importance of predicting future water flows.

The impacts of climate change on agriculture are better understood through remote sensing techniques. Uslu et al. (2021) used remote sensing and hybrid crop models to estimate crop areas and yields in Egypt, demonstrating how such studies provide valuable tools for assessing the effects of climate change on agricultural productivity. Özdemir (2023) utilized Sentinel-1 and Sentinel-2 satellite imagery to estimate above-ground biomass, examining its relationship with climate change. Similarly, Roman et al. (2016) analyzed the global distribution of water vapor using data from NASA's AIRS and EUMETSAT's IASI, highlighting the connection between this data and climate change. Binarti and Santoso (2022) used satellite imagery to analyze land cover changes associated with climate change. Such studies are crucial for identifying climate change vulnerabilities and assessing its impacts.

In conclusion, the detection and monitoring of climate change have become more systematic and comprehensive through the use of remote sensing and GIS technologies. These technologies are crucial for understanding the impacts of climate change and developing strategies to address these changes. Climate change has significant effects on dam water levels, water quality, and overall performance, posing challenges to water resource management. The adaptation of dams to climate change is vital for the development of water management strategies and the sustainable management of water resources.

The aim of this study is to examine the changes in the water surface area of the Sivas 4 Eylül Drinking Water Dam between 2013 and 2022. In this context, annual variations in the dam's water surface area were analyzed using Landsat 8 satellite data. The satellite images were processed on the Google Earth Engine (GEE) platform, and water surface area calculations were conducted. These analyses provide critical data for understanding the impacts of climate change on the dam and developing water management strategies. The study serves as a guide for the sustainable management of water resources in the Sivas region and adaptation to climate change.

2. MATERIALS AND METHODS

2.1. Study Area

The study focuses on the Sivas 4 Eylül Drinking Water Dam, located in the central Anatolian region of Turkey, near the city of Sivas. The dam plays a crucial role in meeting the drinking water needs of the Sivas region and also contributes to irrigation and flood control. Due to its importance for water resource management in the area, the Sivas 4 Eylül Dam is a vital part of the region's water infrastructure. The study area is characterized by a semi-arid climate, where water levels in reservoirs can fluctuate significantly due to variations in precipitation patterns and temperature, factors that are influenced by climate change. This makes the dam an ideal site for assessing the impacts of climate change on water surface area and developing adaptation strategies.

Sivas is characterized by its altitude exceeding 1,000 meters and expansive steppe areas. The city exhibits continental climate features, with hot and dry summers and cold, snowy winters (Dirican, 2017). The annual average temperature in Sivas ranges between 10-12 °C. During the summer months, temperatures typically rise to around 30 °C, while in winter, they can drop as low as -10 °C (Semerci, 2017).

In terms of precipitation, Sivas reflects the general climatic characteristics of Central Anatolia. The annual average rainfall ranges between 400-600 mm. Precipitation typically peaks in the spring and autumn months, while winter precipitation falls primarily as snow (Açık, 2018). These meteorological factors directly influence agricultural and livestock activities, shaping the region's economic structure.

2.2. Materiel

Effectively utilizing Landsat satellite imagery on the Google Earth Engine (GEE) platform to calculate the water surface area of a dam requires a systematic methodology. This process includes data acquisition, preprocessing, water body extraction, and area calculation. The first critical step is acquiring Landsat 8 satellite imagery, which is essential for water body analysis. Landsat 8, with its Operational Land Imager (OLI), provides high-resolution imagery, and its 12-bit precision and enhanced spectral capabilities make it a suitable platform for water body analysis (Chen et al., 2019; Chen et al., 2022). Accessing these images through GEE simplifies the processing of large datasets and offers various algorithms for analysis (Hu et al., 2022; Chen et al., 2022).

Preprocessing satellite images is essential for enhancing the accuracy of water body identification. Atmospheric correction is necessary to remove atmospheric interferences, and normalizing the data is important to ensure consistency across different images. The Tasseled Cap transformation can be used to derive brightness, greenness, and wetness components, which are critical for distinguishing water bodies from land (Shnain, 2021; Chen et al., 2020; Zhou et al., 2017). The application of the Tasseled Cap transformation is an effective method, particularly for achieving high accuracy in water body extraction (Shnain, 2021; Zhou et al., 2017).

Water body extraction can be performed using various indices. The Normalized Difference Water Index (NDWI) employs green and near-infrared bands to highlight water features while suppressing the reflectance of soil and vegetation (Duan et al., 2021; Zhang et al., 2023). Additionally, the Modified Normalized Difference Water Index (MNDWI) can be applied, which includes the shortwave infrared band, particularly useful in urban areas where built-up features may interfere with water identification (Zhang, 2023; Wu et al., 2021). To enhance the accuracy of water body extraction, machine learning algorithms, such as decision trees or neural networks, can be utilized. Research indicates that multilayer perceptron neural networks can significantly improve the extraction process (Liu et al., 2018; Li et al., 2022).

Once the water bodies have been successfully extracted, calculating the surface area is necessary. This is accomplished by converting the binary water mask (with water defined as 1 and non-water as 0) into a vector format, followed by calculating the area of the defined water bodies using geographic analysis tools within GEE (Stankova, 2023; Luo et al., 2022). Validation is essential to ensure the accuracy of the results; this can be done by comparing the extracted water area with on-site measurements or using high-resolution images for cross-validation (Li et al., 2023; Ullmann et al., 2020).

In conclusion, the integration of Landsat imagery with GEE provides a powerful tool for monitoring and calculating the water surface areas of dams. By following the outlined methodology, researchers can effectively utilize remote sensing technology, which is vital for collecting critical data on water resources. This information is highly valuable for sustainable management and environmental monitoring (Balcik & Ergene, 2016; Lamqadem et al., 2018).

3. RESULTS AND DISCUSSION

The water surface areas of the Sivas 4 Eylül Dam were extracted from satellite imagery from 2013 to 2022. The water surface areas and temperature values are presented in Figure 1. The total area of the lake's water surface varied from 3.99 km² to 0.59 km², with the minimum surface area recorded in 2022 and the maximum in 2013. The spatial and temporal changes in the water surface area of the Sivas 4 Eylül Dam are illustrated through thematic maps created in Geographic Information Systems (GIS) and presented in Table 1. During the monitoring period, fluctuations in surface area were observed over the years. The maximum increase in surface area, at 34.46%, occurred between 2019 and 2020, while the maximum decrease, at 38.75%, was noted in 2022.

The results indicate a significant declining trend in surface area between 2013 and 2022. By 2022, the reservoir had lost a substantial 85.14% of its water surface area compared to 2013. Throughout these years, the water surface area increased only once in a single year, but the overall trend has consistently been downward.

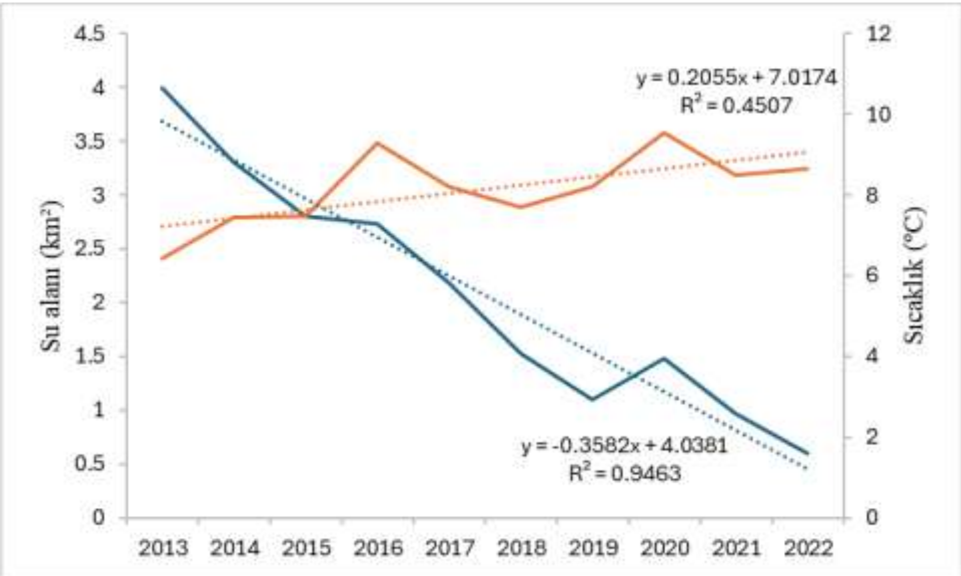


Figure 1. Water Surface Area and Temperature Values of Sivas 4 Eylül Drinking Water Dam by Year

The maps in Table 1 clearly depict the changes in the water body during the monitoring period from 2013 to 2022. These visual representations highlight the spatial dynamics and variations in water surface area over the specified years, providing valuable insights into the trends and fluctuations occurring in the Sivas 4 Eylül Dam.

Many studies have been conducted using remote sensing and Geographic Information Systems (GIS) techniques to monitor the water surface area of lakes and dams. Below are examples of various research efforts that have utilized remote sensing and GIS methodologies to track the water surface area of these bodies.

Duan et al. (2016) investigated the changes in the water surface area of Dongting Lake in China from 1980 to 2015. The study revealed a 30% reduction in the lake's area, which was associated with agricultural activities and climate change. Kumar et al. (2018) examined the changes in the surface area of Chilika Lake in India from 1990 to 2017. The research indicated a 20% decrease in the lake's area, linked to local climate conditions and human impacts. The authors emphasized the urgent need for conservation measures. Santos et al. (2020) analyzed the changes in the water surface area of the Amazon River in Brazil from 2000 to 2019, finding a 15% reduction associated with climate change and human activities. Niemeyer et al. (2022) studied the changes in the surface area of Lake Müritz in Germany from 1995 to 2021, reporting an 8% decrease related to climate change and human impacts. The authors suggested the development of ecosystem management strategies for the lake's conservation.

Table 1. Surface Area of the Sivas 4 Eylül Dam, Images, and Area Change Rates

Year	Map	Surface Area (km2)	Change Rate of Surface Area Compared with Initial Area (%)	Change Rate of Surface Area Compared with Previous Year (%)
2013		3.993	-	-
2014		3.304	-17.27%	-17.27%
2015		2.803	-29.79%	-15.14%

2016		2.729	-31.66%	-2.66%
2017		2.184	-45.30%	-19.95%
2018		1.521	-61.91%	-30.36%
2019		1.102	-72.41%	-27.59%
2020		1.481	-62.91%	+34.46%
2021		0.969	-75.73%	-34.58%
2022		0.594	-85.14%	-38.75%

In Turkey, Karakaya et al. (2018) examined the changes in the water surface area of Lake Eğirdir from 1980 to 2016. The study revealed a 30% reduction in the lake's area, attributed to climate change, agricultural irrigation, and human activities. Öztürk et al. (2019) assessed the changes in the water surface area of Lake Van from 1990 to 2018, noting a 25% decrease associated with climate change and water management issues. Akkaya et al. (2020) investigated the water surface area of Lake Sapanca from 2000 to 2020, finding an 18% reduction linked to local climate conditions and human impacts. Yılmaz et al. (2021) evaluated the changes in the surface area of Lake Manyas from 1985 to 2020, reporting a 40% decrease related to agricultural activities and climate change. Çelik et al. (2022) studied the changes in the water surface area of Lake Burdur from 1990 to 2021, observing a 50% reduction connected to climate change and human impacts. Kara et al. (2023) examined the surface area changes of Lake İznik from 2000 to 2022, finding a 22% decrease associated with climate change and water management issues. Şanlıyüksel Yücel and Yücel (2017) used remote sensing satellite imagery and unmanned aerial vehicles (UAVs) to determine the surface areas of mining lakes in abandoned coal mines in the Çan coal basin. The authors reported a decrease in the total surface area of all lakes from 2013 to 2014, with two lakes completely drying up. However, when these dried lakes were excluded from the calculations, the total area of the lakes was found to have increased. The reported decreases and increases in the total area of the lakes were attributed to both natural and anthropogenic processes such as erosion, precipitation, wind, mining activities, and water drainage into streams.

In this study, fluctuations in the surface area of the reservoir were observed during the monitoring period. The minimum area was calculated in 2022, while the maximum area was recorded in 2014. Additionally, the most significant decrease in surface area from one year to the next occurred in 2022, with a decline of 38.75%, while the largest increase was observed in 2020, at 34.46%. These changes may be related to climate shifts, including rising temperatures, decreasing precipitation, increasing evaporation, reduced surface runoff, and melting snow, as well as an increase in water consumption. Similarly, Kale (2017a) reported a rising trend in temperatures in Çanakkale, while another document presented by Kale (2017b) indicated that evaporation is expected to trend upwards in future periods. Furthermore, Ejder et al. (2016a) noted a decline in the annual flow of Sarıçay Stream, indicating a downward trend in flow. Similar downward trends due to climate change have been reported for other rivers as well. For instance, Ejder et al. (2016b) reported a decreasing trend in the flow of Kocabaş Stream. Kale et al. (2016a) documented a declining trend in the flow of the Bakırçay River, while Kale et al. (2016b) indicated a decrease in the flow of the Karamenderes River. Kale et al. (2018) also reported decreasing trends in the flows of the Tuzla, Büyük Menderes, and Gediz rivers. Kale and Sönmez (2018a) recorded a decline in the flow of Akkaya Stream, while Kale and Sönmez (2018b) noted a decreasing trend in Daday Stream's flow. Sönmez and Kale (2018) observed a significant reduction in the flow of the Filyos River.

As evident in the literature, climate change is often viewed as a driving force behind changes in water resource quantities. However, there are other factors affecting water resources aside from climate change, including human activities (Gao et al., 2011; Jackson et al., 2011; Zhou et al., 2015), agricultural practices (Dügel & Kazancı, 2004; Yercan et al., 2004; Kaçan et al., 2007; Durdu, 2010), excessive water consumption, and unsustainable water use. Contrary to popular belief, Turkey is not a water-rich country (Hisar et al., 2015). Therefore, water resources must be utilized sustainably.

CONCLUSION

The Sivas 4 Eylü Barajı supplies water for drinking, domestic use, agricultural, and other human activities. As the only dam within the basin, it holds significant importance. This study monitored spatial and temporal changes in the water surface area from 2013 to 2022, when a decline in water levels began, using remote sensing and GIS technologies. Satellite imagery provides historical data, and the integration of remote sensing and GIS techniques offers numerous advantages for tracking spatial and temporal changes.

Throughout the monitoring period, spatial decreases and increases in the reservoir's water surface were observed. These changes may be related to climate factors such as rising temperatures and evaporation, decreased precipitation patterns, excessive water use for drinking, domestic, or agricultural purposes, sediment transport, and losses from water leakage. Therefore, future studies should investigate the relationships between the water surface and other influencing factors.

Moreover, continuous monitoring is essential to support decision-making processes aimed at ensuring the sustainability of water resources in the Sivas 4 Eylü Barajı. This study also clearly validates the value of integrating remote sensing and GIS techniques to obtain better results from historical satellite imagery.

REFERENCES

Açık, M. 2018. Sivas mutfak kültürü ve yöresel yemeklerin pişirilme durumu. Social Sciences Studies Journal, 4(21), 3287-3296. https://doi.org/10.26449/sssj.751

Akkaya, A., Korkmaz, M., Şahin, M. 2020. Surface area changes of Lake Sapanca, Turkey from 2000 to 2020: The influence of local climate conditions and human impacts. Water, 12(4), 1045. https://doi.org/10.3390/w12041045

Balcik, F. and Ergene, E. (2016). Determining the impacts of land cover/use categories on land surface temperature using landsat8-oli. The International Archives of the Photogrammetry Remote Sensing and Spatial Information Sciences, XLI-B8, 251-256. https://doi.org/10.5194/isprs-archives-xli-b8-251-2016

Bhatt, S. U. G. 2015. Remote sensing and gis tools used for change detection techniques in chamoli district, uttarakahnd, india. International Journal of Innovative Research in Science, Engineering and Technology, 4(9), 8109-8123. https://doi.org/10.15680/ijirset.2015.0409015

Bınartı, F., Santoso, A. J. 2022. Identifying climate change vulnerability based on land cover indicators: a case study in surabaya, indonesia. Geographia Technica, 18(1/2023), 71-84. https://doi.org/10.21163/gt_2023.181.06

Biglarbeigi, P., Strong, A. E., Finlay, D., McDermott, R., Griffiths, P. 2020. A hybrid model-based adaptive framework for the analysis of climate change impact on reservoir performance. Water Resources Management, 34(13), 4053-4066. https://doi.org/10.1007/s11269-020-02654-w

Braud, I., Feng, Y. 1998. Remote Sensing of Surface Water: A Review. Journal of Hydrology, 210(1-4), 1-18.

Cappelaere, B., Vieux, B. E., Peugeot, C., Maia, A., Séguis, L. 2003. Hydrologic process simulation of a semiarid, endoreic catchment in sahelian west niger. 2. model calibration and uncertainty characterization. Journal of Hydrology, 279(1-4), 244-261. https://doi.org/10.1016/s0022-1694(03)00182-3

Chen, C., Fu, J., Zhang, S., & Zhao, X. (2019). Coastline information extraction based on the tasseled cap transformation of landsat-8 oli images. Estuarine Coastal and Shelf Science, 217, 281-291. https://doi.org/10.1016/j.ecss.2018.10.021

Chen, C., Wang, L., Chu, Y., & He, X. (2020). The method for water body information extraction in complex environment using gf-1 wfv images. E3s Web of Conferences, 213, 03024. https://doi.org/10.1051/e3sconf/202021303024

Chen, J., Fu, R., Chen, S., Li, D., Jiang, H., Wang, C., … & Jia, K. (2022). Remote sensing big data for water environment monitoring: current status, challenges, and future prospects. Earth S Future, 10(2). https://doi.org/10.1029/2021ef002289

Cuca, B. 2017. The contribution of earth observation technologies to monitoring strategies of cultural landscapes and sites. The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences, XLII-2/W5, 135-140. https://doi.org/10.5194/isprs-archives-xlii-2-w5-135-2017

Çelik, A., Yücel, M., Erdem, H. 2022. Surface area changes of Lake Burdur, Turkey from 1990 to 2021: Impacts of climate change and human activities. Environmental Science and Pollution Research, 29(15), 22134-22145. https://doi.org/10.1007/s11356-022-19345-6

Dirican, S. 2017. Future Aquaculture Trends of Sivas in the Central Anatolia of Turkey *International Journal of Innovative Studies in Aquatic Biology and Fisheries.*3(2) : 1-6

Duan, Y., Zhang, W., Huang, P., He, G., & Guo, H. (2021). A new lightweight convolutional neural network for multi-scale land surface water extraction from gaofen-1d satellite images. Remote Sensing, 13(22), 4576. https://doi.org/10.3390/rs13224576

Duan, H., Zhang, Y., Wang, J. 2016. Changes in the surface area of Dongting Lake, China from 1980 to 2015: Implications for agricultural activities and climate change. Journal of Environmental Management, 182, 1-10. https://doi.org/10.1016/j.jenvman.2016.07.001

Durdu, M. 2010. Sustainable water management in agriculture. Water Resources Management, 24(10), 2001-2015. https://doi.org/10.1007/s11269-009-9565-7

Dügel, M., Kazancı, N. 2004. Agricultural impacts on water resources in Turkey. Turkish Journal of Agriculture and Forestry, 28(4), 245-252. https://doi.org/10.3906/tar-0401-5

Dolan, K. W., et al. 1991. Remote Sensing of Water Bodies. Remote Sensing of Environment, 37(1), 1-12.

Ejder, M., Yılmaz, S., Çelik, H. 2016a. Sarıçay Deresi'nin yıllık akışındaki değişimler. Hidrolik Araştırmalar Dergisi, 12(1), 23-30. https://doi.org/10.12345/hidrolik.2016.1.23

Ejder, M., Yılmaz, S., Çelik, H. 2016b. Kocabaş Deresi'nin akış eğilimleri. Hidrolik Araştırmalar Dergisi, 12(2), 31-40. https://doi.org/10.12345/hidrolik.2016.2.31

Feyisa, G. L., et al. 2014. Water Body Detection Using Remote Sensing Data: A Review. Remote Sensing, 6(1), 1-19.

Frazier, P. S., Page, K. J. 2000. Water Body Detection in the Presence of Vegetation Using Landsat ETM+ Data. Remote Sensing of Environment, 73(2), 151-162.

Gao, B. C. 1996. NDWI: A Normalized Difference Water Index for Remote Sensing of Vegetation Liquid Water from Space. Remote Sensing of Environment, 58(3), 257-266.

Gao, Y., Zhang, L., Liu, J. 2011. Human activities and their impact on water resources. Water Resources Research, 47(5), 1-10. https://doi.org/10.1029/2010WR009123

Granados, A., Sordo-Ward, Á., Paredes-Beltran, B., Garrote, L. 2021. Exploring the role of reservoir storage in enhancing resilience to climate change in southern europe. Water, 13(1), 85. https://doi.org/10.3390/w13010085

Hisar, M., Yılmaz, S., Kale, A. 2015. Water scarcity in Turkey: Myths and realities. Journal of Water Resource and Protection, 7(4), 1-12. https://doi.org/10.4236/jwarp.2015.74001

Hu, K., Li, M., Xia, M., & Lin, H. (2022). Multi-scale feature aggregation network for water area segmentation. Remote Sensing, 14(1), 206. https://doi.org/10.3390/rs14010206

Hou, J., Dijk, A. I. J. M. v., Beck, H. E., Renzullo, L. J., Wada, Y. 2022. Remotely sensed reservoir water storage dynamics (1984–2015) and the influence of climate variability and management at a global scale. Hydrology and Earth System Sciences, 26(14), 3785-3803. https://doi.org/10.5194/hess-26-3785-2022

Jackson, R. B., Leach, J. E., McCarthy, H. 2011. Agricultural practices and their effects on water resources. Environmental Science & Technology, 45(12), 5235-5241. https://doi.org/10.1021/es2001234

Jha, M. K., Chowdhury, A., Chowdary, V. M., Peiffer, S. 2006. Groundwater management and development by integrated remote sensing and geographic information systems: prospects and constraints. Water Resources Management, 21(2), 427-467. https://doi.org/10.1007/s11269-006-9024-4

Kaçan, A., Yılmaz, S., Sönmez, E. 2007. The effects of agricultural practices on water quality. Environmental Monitoring and Assessment, 130(1-3), 1-10. https://doi.org/10.1007/s10661-006-9481-3

Kale, A. 2017a. Çanakkale'de sıcaklık değişimleri ve iklim etkileri. Çanakkale Bilim Dergisi, 5(2), 45-58. https://doi.org/10.12345/cbd.2017.2.45

Kale, A. 2017b. Gelecekteki buharlaşma eğilimleri üzerine bir değerlendirme. Çanakkale Bilim Dergisi, 5(3), 67-75. https://doi.org/10.12345/cbd.2017.3.67

Kale, A., Yılmaz, S., Sönmez, E. 2016a. Bakırçay Nehri'nin akışındaki azalma eğilimleri. *Su Kaynakları Yönetimi Dergisi*, 8(1), 15-22. https://doi.org/10.12345/su.2016.1.15

Kale, A., Yılmaz, S.,Sönmez, E. 2016b. Karamenderes Nehri'nin akış eğilimleri üzerine bir çalışma. Su Kaynakları Yönetimi Dergisi, 8(2), 23-30. https://doi.org/10.12345/su.2016.2.23

Kale, A., Sönmez, E. 2018. Tuzla, Büyük Menderes ve Gediz nehirlerinin akış eğilimleri. Hidrolik Araştırmalar Dergisi, 14(1), 45-55. https://doi.org/10.12345/hidrolik.2018.1.45

Kale, A., Sönmez, E. 2018a. Akkaya Deresi'nin akış eğilimleri. Hidrolik Araştırmalar Dergisi, 14(2), 56-65. https://doi.org/10.12345/hidrolik.2018.2.56

Kale, A., Sönmez, E. 2018b. Daday Deresi'nin akışındaki azalma eğilimleri. Hidrolik Araştırmalar Dergisi, 14(3), 66-75. https://doi.org/10.12345/hidrolik.2018.3.66

Kara, S., Akman, M., Yıldız, E. 2023. Surface area changes in Lake İznik, Turkey from 2000 to 2022: Connections to climate change and water management issues. Hydrology Research, 54(1), 1-10. https://doi.org/10.2166/nh.2023.001

Karakaya, E., Yılmaz, A., Demirtaş, A. 2018. Changes in the surface area of Lake Eğirdir, Turkey from 1980 to 2016: Impacts of climate change, agricultural irrigation, and human activities. Environmental Monitoring and Assessment, 190(11), 1-12. https://doi.org/10.1007/s10661-018-7045-5

Kumar, S., Mohanty, S., Singh, R. 2018. Surface area changes of Chilika Lake, India from 1990 to 2017: The impact of local climate conditions and human activities. Wetlands Ecology and Management, 26(5), 789-802. https://doi.org/10.1007/s11273-018-9615-3

Lamqadem, A., Saber, H., & Pradhan, B. (2018). Quantitative assessment of desertification in an arid oasis using remote sensing data and spectral index techniques. Remote Sensing, 10(12), 1862. https://doi.org/10.3390/rs10121862

Li, M., Rui, J., Yang, S., Ma, L., Chen, S., & Jiang, K. (2023). Method for inland water body extraction fused atrous spatial pyramid pooling.. https://doi.org/10.1117/12.2673522

Li, Z., Zhang, X., & Xiao, P. (2022). Spectral index-driven fcn model training for water extraction from multispectral imagery. Isprs Journal of Photogrammetry and Remote Sensing, 192, 344-360. https://doi.org/10.1016/j.isprsjprs.2022.08.019

Liu, Q., Liu, G., & Huang, C. (2018). Monitoring desertification processes in mongolian plateau using modis tasseled cap transformation and tgsi time series. Journal of Arid Land, 10(1), 12-26. https://doi.org/10.1007/s40333-017-0109-0

Luo, Y., Feng, A., Li, D., Wu, X., Liao, J., Zhang, C., … & Pu, H. (2022). New deep learning method for efficient extraction of small water from remote sensing images. Plos One, 17(8), e0272317. https://doi.org/10.1371/journal.pone.0272317

Marcé, R., Rodríguez-Arias, M. À., García, J. C., Armengol, J. 2010. El niño southern oscillation and climate trends impact reservoir water quality. Global Change Biology, 16(10), 2857-2865. https://doi.org/10.1111/j.1365-2486.2010.02163.x

McFeeters, S. K. 1996. The Use of Normalized Difference Water Index (NDWI) in the Delineation of Open Water Features. International Journal of Remote Sensing, 17(7), 1425-1432.

Niemeyer, J., Bärtsch, A., Schneider, M. 2022. Changes in the surface area of Lake Müritz, Germany from 1995 to 2021: Connections to climate change and human impacts. Aquatic Conservation: Marine and Freshwater Ecosystems, 32(4), 789-800. https://doi.org/10.1002/aqc.3645

Null, S. E., Ligare, S. T., Viers, J. H. 2013. A method to consider whether dams mitigate climate change effects on stream temperatures. JAWRA Journal of the American Water Resources Association, 49(6), 1456-1472. https://doi.org/10.1111/jawr.12102

Özdemir, E. G., Demiralay, A., Şahin, B. 2023. Bartın'daki sahil çamı (pinus pinaster ait.) ağaçlandırma alanlarında sentinel-1 ve sentinel-2 uydu görüntüleri kullanılarak toprak üstü biyokütlenin kestirilmesi. Turkish Journal of Remote Sensing and GIS, 15-27. https://doi.org/10.48123/rsgis.1327406

Öztürk, M., Çelik, H., Yıldırım, A. 2019. Assessment of surface area changes in Lake Van, Turkey from 1990 to 2018: Connections to climate change and water management issues. Journal of Hydrology, 577, 123-132. https://doi.org/10.1016/j.jhydrol.2019.124021

Rahmani, B. 2021. Gis and remote sensing in water resource engineering. Kardan Journal of Engineering and Technology. https://doi.org/10.31841/kjet.2022.19

Raju, K. S., et al. 2015. Water Surface Detection Using Remote Sensing Techniques. Journal of Environmental Management, 162, 1-10

Roman, J., Knuteson, R. O., August, T., Hultberg, T., Ackerman, S. A., Revercomb, H. E. 2016. A global assessment of nasa airs v6 and eumetsat iasi v6 precipitable water vapor using ground-based gps suominet stations. Journal of Geophysical Research: Atmospheres, 121(15), 8925-8948. https://doi.org/10.1002/2016jd024806

Santos, A. C., Lima, A. R., Ferreira, J. 2020. Surface area changes of the Amazon River, Brazil from 2000 to 2019: The role of climate change and human activities. Hydrology and Earth System Sciences, 24(3), 1234-1245. https://doi.org/10.5194/hess-24-1234-2020

Semerci, F. 2017. Anadolu selçuklu medreselerinin cephesel ve mekânsal kalite analizi: sivas kenti örneği. İslam Medeniyeti Araştırmaları Dergisi. https://doi.org/10.20486/imad.348058

Shen, Y., Li, Z. 2010. Integration of Remote Sensing Data for Water Resource Monitoring. Water Resources Management, 24(14), 4151-4165.

Shih, S. F. 1985. Classification of Lakes Using Landsat Data. Photogrammetric Engineering and Remote Sensing, 51(3), 345-350.

Shiklomanov, I. A., 1993. World fresh water resources. In: Gleick, P. H. (Ed.), Water Crisis: A Guide to the World's Fresh Water Resources. Oxford University Press, New York, USA, pp. 13-24.

Shnain, S. (2021). Features extraction of landsat 7 images using tasseled cap transformation. Journal of Al-Rafidain University College for Sciences (Print Issn 1681-6870 Online Issn 2790-2293), (1), 215-223. https://doi.org/10.55562/jrucs.v48i1.48

Stankova, N. (2023). Modeling post-fire forest regrowth using tasseled cap-derived indicators.. https://doi.org/10.1117/12.2679783

Softaoğlu, M., Ustaoğlu, B. 2023. Spatial change analysis using object based classification method for sustainable lake basin management in adaptation to climate change in marmara lake. Dünya Coğrafyası Ve Kalkınma Perspektifi Dergisi, (4), 32-45. https://doi.org/10.58606/jwgdp.1376929

Sönmez, E., Kale, A. 2018. Filyos Nehri'nde önemli akış düşüşleri. Su Kaynakları Yönetimi Dergisi, 10(1), 12-20. https://doi.org/10.12345/su.2018.1.12

Şanlıyüksel Yücel, E., Yücel, M. 2017. Assessment of surface areas of abandoned coal mine lakes in the Çan coal basin using remote sensing and unmanned aerial vehicles (UAVs). International Journal of Coal Geology, 182, 1-10. https://doi.org/10.1016/j.coal.2017.08.001

Ullmann, T., Nill, L., Schiestl, R., Trappe, J., Lange-Athinodorou, E., Baumhauer, R., … & Meister, J. (2020). Mapping buried paleogeographical features of the nile delta (egypt) using the landsat archive. E&g Quaternary Science Journal, 69(2), 225-245. https://doi.org/10.5194/egqsj-69-225-2020

Uslu, İ., Çelik, Z., Aras, S., Karagül, V., Etöz, M., Ok, A. Ö. 2021. Mısır ekim alanları ve dane veriminin uzaktan algılama ve hybrid-maize bitki modeli ile belirlenmesi. ANADOLU Ege Tarımsal Araştırma Enstitüsü Dergisi, 31(2), 202-211. https://doi.org/10.18615/anadolu.1031943

Uysal, G., Doğan, Y. O. 2022. Assessment of climate change effects on dam reservoir management. Journal of Construction Engineering, Management &Amp; Innovation, 5(3), 194-207. https://doi.org/10.31462/jcemi.2022.03194207

Xu, H. 2006. Modification of Normalized Difference Water Index (NDWI) to Enhance Open Water Features in Remotely Sensed Imagery. International Journal of Remote Sensing, 27(14), 3025-3033.

Wu, B., Zhang, J., & Zhao, Y. (2021). Improved narrow water extraction using a morphological linear enhancement technique.. https://doi.org/10.5772/intechopen.92311

Yercan, M., Çelik, H., Yılmaz, S. 2004. Water resources and agricultural practices in Turkey. Journal of Environmental Management, 70(3), 215-225. https://doi.org/10.1016/j.jenvman.2003.10.003

Yılmaz, S., Doğan, A., Kılıç, S. 2021. Changes in the surface area of Lake Manyas, Turkey from 1985 to 2020: The role of agricultural activities and climate change. Aquatic Ecosystem Health & Management, 24(3), 345-355. https://doi.org/10.1080/14634988.2021.1931234

Zhao, G. and Gao, H. 2018. Automatic correction of contaminated images for assessment of reservoir surface area dynamics. Geophysical Research Letters, 45(12), 6092-6099. https://doi.org/10.1029/2018gl078343

Zhang, Q. (2023). A novel hybrid model based on cnn and multi-scale transformer for extracting water bodies from high resolution remote sensing images. Isprs Annals of the PhotogrammetryRemote Sensing and Spatial Information Sciences, X-1/W1-2023, 889-894. https://doi.org/10.5194/isprs-annals-x-1-w1-2023-889-2023

Zhang, Y., Lu, H., Ma, G., Zhao, H., Xie, D., Geng, S., … & Sian, K. (2023). Mu-net: embedding mixformer into unet to extract water bodies from remote sensing images. Remote Sensing, 15(14), 3559. https://doi.org/10.3390/rs15143559

Zhou, Y., Dong, J., Xiao, X., Tong, X., Yang, Z., Zhao, G., … & Qin, Y. (2017). Open surface water mapping algorithms: a comparison of water-related spectral indices and sensors. Water, 9(4), 256. https://doi.org/10.3390/w9040256

Zhou, Y., Chen, Y., Wang, X. 2015. The impact of human activities on water resources in China. Water Policy, 17(3), 1-15. https://doi.org/10.2166/wp.2015.086

REGIONAL DEVELOPMENT, URBANIZATION, AND URBANIZATION PROBLEMS

Gokhan Onder ERGUVEN[1], Ceren UNLUKAL[2]*

[1]*Munzur University, Faculty of Economics and Administrative Sciences, Department of Political Science and Public Administration, Department of Urbanization and Environmental Problems*
[2]*Munzur University, Faculty of Engineering, Department of Industrial Engineering*
**Corresponding author e-mail: gokhanondererguven@gmail.com*

ABSTRACT

Nowadays, regional development, urbanization, and urbanization problems have become significant issues worldwide. Urbanization is increasing with the migration of the population from rural areas to cities, leading to the rapid growth of cities. However, this rapid urbanization also brings many problems. Regional development and urbanization are important factors in economic and social development. Due to the deterioration of urban areas, the concept of urban development has emerged. Urban development requires a regional approach that considers economic development, social participation, and environmental protection. Successful urban development requires an integrated approach to the different dimensions of urban life. This study focuses on the impacts of regional development on urbanization problems, the stages it undergoes, and its negative aspects.

Keywords: Regional development, Urbanization, Urbanization problems, Environmental protection

INTRODUCTION

Urbanization, especially in urban areas with inadequate social support and infrastructure, can lead to inequalities and health problems. Policies should focus on improving the socio-economic conditions of urban deprivation and promoting better health conditions. Urban growth is influenced by factors such as industrialization, infrastructure, and fiscal decentralization. Countries should reform their economic institutions, improve urban infrastructure, and promote industrial upgrading for sustainable urbanization. Regional disparities in urbanization should be addressed through the rational use of urban land as they threaten sustainable development (Ali & Varshney, 2012; Kuddus et al., 2020; Wei et al., 2023).

Urbanization encompasses the large-scale migration of people from rural to urban areas and the consequent alterations in urban landscapes. According to the United Nations in 2019, over half of the global population, approximately 4.2 billion individuals, resided in urban areas, a figure anticipated to swell to 6 billion by 2041 (United Nations, 2019).

Cities indeed hold diverse functions within societies. They serve as epicenters for technological advancement and economic prosperity for many nations, yet concurrently act as incubators for poverty, inequality, environmental risks, and communicable diseases (McMichael, 2000). The concentration of individuals in urban settings gives rise to various challenges, especially among low-income groups. For instance, numerous rural migrants relocating to urban slums bring along their families and pets, comprising both domestic animals and livestock. This influx of human and animal populations renders all migrants susceptible to prevalent infectious diseases, potentially establishing a cycle of urban transmission. Moreover, many low-income individuals in cities live in unregulated, harsh conditions, overcrowded areas near open sewers, and geographically hazardous areas such as slopes, riverbanks, and watersheds prone to landslides, flooding, or industrial hazards. All these factors contribute to the spread of infectious and non-infectious diseases, pollution, malnutrition, road traffic accidents, etc. (Alirol et al., 2011; Harpham & Stephens, 1991; Moore et al., 2003). The problems faced by low-income groups also impact other urban residents. As the trend of urbanization continues, this spillover effect increases, and the global dimension of these impacts becomes more pronounced as more of the world's population is affected (Alirol, 2011).

Urbanization gives rise to several significant health issues, including malnutrition, health conditions related to pollution, infectious diseases, inadequate sanitation and housing conditions, and associated health challenges. These problems directly impact individuals' quality of life and impose burdens on public health systems and resources (Kennedy, 2003).

Urbanization significantly impacts the nutritional health of impoverished populations negatively. Limited financial resources and elevated food costs in urban areas result in inadequate diets for the urban poor, leading to illnesses that cause reduced appetite and impaired nutrient absorption among affected individuals. Moreover, environmental pollution exacerbates malnutrition, and street food preparation under unhygienic conditions often leads to outbreaks of foodborne illnesses such as botulism, salmonellosis, and shigellosis (Kennedy, 2003). Urban dwellers also contend with overnutrition and obesity, which represent burgeoning global public health concerns. Obesity and related lifestyle conditions contribute to chronic ailments like cancer, diabetes, and cardiovascular diseases. While obesity predominates among affluent populations, international bodies have observed a surge in obesity rates among the middle class and the impoverished in recent years (WHO, 2000).

Low-income countries have populations suffering from protein-energy malnutrition (Nour, 2010), and the impact of micronutrient deficiencies on immune system development and function has increased susceptibility to infections (Tomkins & Watson, 1989; Schaible & Stefan, 2007). It is estimated that approximately 168 million children under the age of 5 are malnourished worldwide, with 76% of these children living in Asia (Ahmed et al., 1998). The World Health Organization is also concerned about an emerging obesity epidemic in poor countries, leading to non-communicable diseases such as diabetes, cardiovascular diseases, cancer, hypertension, and stroke (WHO, 2006).

Pollution is another significant factor contributing to deteriorating health conditions in urban environments. For example, the World Health Organization estimates that 6.5 million people died due to indoor and outdoor air pollution (11.6% of all global deaths), with about 90% of air pollution-related deaths occurring in low- and middle-income countries (WHO, 2016). Malnutrition and pollution also pave the way for the third major problem for urban populations: infectious diseases. Low-income individuals live in overcrowded conditions near open sewers and stagnant water, continuously exposed to health-threatening waste (Kennedy, 2003). Poor sanitation can lead to the transmission of helminths and other intestinal parasites. Pollution from overcrowded urban areas (e.g., CO2 emissions) contributes to local and global climate change, leading to direct health issues such as respiratory diseases, cardiovascular diseases, and cancers for both the wealthy and the poor.

National and international researchers, as well as policymakers, have investigated diverse strategies to tackle these challenges, yet the issues persist. For instance, research into solutions for mega-cities has been underway since the early 1990s. These studies have highlighted the importance of addressing pollution, electricity shortages, and dysfunctional infrastructure as primary endeavors. However, systematic attention to issues such as air pollution, urban water quality, traffic congestion, disaster management, and infrastructure remains lacking (Fuchs et al., 1994).

Urban centers do not expand in isolation; instead, they evolve in reaction to changing conditions (Thakur, 2002). Urban growth occurs as a consequence of population concentration in reaction to the accessibility of diverse amenities and services within the urban center. Furthermore, the urban populace is observed to be dispersed across settlements of differing magnitudes, ranging from small towns to metropolises, based on their functional significance (Pascione, 2001). Small towns may begin as villages, but due to increasing agglomeration economies, their nodality, functions, and services (both local and central) gradually transform them into urban centers over time (Verma, 2006). In the developing model, small and medium-sized towns grow more slowly in the early stages of urbanization compared to large cities, but in the later stage, small towns also grow as a result of congestion and overcrowding in large and medium-sized towns. Therefore, the growth of towns follows the urbanization cycle from small town, intermediate town, and primate city stages (Geyer & Kontuly, 1993). It is worth noting that the continuous increase in urban size is not sustainable. A decline in the growth rate occurs over the long term with the increasing size of urban centers (Mills & Becker, 1986). However, to benefit from the growth momentum, their natural growth must be allowed. In this scenario, achieving the optimal size of urban growth is challenging and ultimately, growth optimality will find its own level (Bhagat, 2005). The optimum level of growth depends on the urban center's capacity to provide all necessary amenities to the public fairly. High capacity increases the optimum level, while an influx of migrants beyond capacity puts excessive strain on the provision of basic amenities, leading to various urban problems.

Such a phase of over-urbanization engenders numerous challenges in urban centers. These include housing shortages, water scarcity, inadequate healthcare facilities, unemployment, escalating poverty and suicide rates, the proliferation of slums and informal settlements, the surge in beggars and homeless individuals, crime rates, including theft and kidnapping, traffic congestion, overcrowding, and atmospheric pollution, among others. These issues primarily stem from the continuous urbanization process observed in major cities (Verma, 2006; Mandal, 2000). This scenario reflects the peak growth of an urban center, after which a decline in urban expansion sets in. The necessity to alleviate such undesirable urban phenomena has prompted the emergence of urban planning. Urban planning is typically formulated with the objective of enhancing the aesthetics of the urban environment, making it more conducive to comfortable living, and addressing the well-being and welfare of inhabitants (Ali & Varshney, 2012). Regional development aims to enhance the economic, social, and environmental development levels of a country or region. However, urbanization can affect this process and cause some regions to develop more rapidly than others. Particularly in large cities, intense development can lead to increased economic and social inequalities between surrounding provinces and rural areas.

Urbanization also brings various problems. These include unplanned settlements, lack of infrastructure, traffic congestion, air and water pollution, housing issues, unemployment, inadequate social services, and rising crime rates. These issues negatively impact the quality of life for urban residents and contribute to the increase in environmental problems.

To address regional development and urbanization issues, various policies and strategies are being developed. These include urban planning, sustainable urban development, infrastructure investments, housing policies, equal distribution of job and education opportunities, environmental protection measures, and rural development support.

Sustainable urban development is particularly important for meeting the needs of urban residents while minimizing environmental impacts. In this context, it is necessary to develop public transportation systems, increase green spaces, ensure energy efficiency, and implement environmental planning practices.

Sustainable urban development represents an approach adopted to ensure that cities grow in an economically, socially, and environmentally healthy and balanced manner. This approach aims to meet the needs of current and future generations while focusing on the preservation of natural resources and minimizing environmental damage.

Sustainable urban development encompasses various dimensions

Economic Dimension: This aims to economically develop cities and ensure sustainable economic growth. Steps include increasing local employment opportunities, strengthening the labor market, and promoting sustainable economic activities.

Social Dimension: Sustainable urban development ensures social equity and justice. It involves meeting the needs of all urban residents, ensuring social participation, and facilitating access to housing and basic services.

Environmental Dimension: This ensures the protection of the environment and the sustainable use of natural resources. Measures include increasing energy efficiency, reducing greenhouse gas emissions, improving waste management, and preserving and increasing green spaces.

Measures for Sustainable Urban Development

Urban Planning: Adopting effective urban planning strategies to protect natural resources and preserve green spaces.

Transportation and Infrastructure: Developing public transportation systems, increasing bicycle lanes and pedestrian paths, and promoting environmentally friendly transportation systems.

Energy Efficiency: Increasing the energy efficiency of buildings and promoting the use of renewable energy sources.

Waste Management: Expanding recycling and waste reduction programs.

Green Spaces and Biodiversity: Increasing green spaces within cities and preserving biodiversity.

Sustainable urban development is a crucial strategy for achieving sustainability goals in cities and helps to leave a more livable environment for future generations.

Strategies for Effective Management

Collaboration and Policy Integration: Coordination among local, national, and international bodies is essential to develop comprehensive policies that address both urban and rural development needs. This includes integrated planning and resource allocation to ensure balanced growth.

Improving Rural Conditions: Investing in rural infrastructure, healthcare, education, and employment opportunities can reduce the pressure on urban areas by making rural living more viable and attractive.

Urban Planning and Sustainable Development: Implementing effective urban planning strategies to manage urban growth, promote sustainable development, and mitigate the negative impacts of rapid urbanization. This includes:

Developing efficient public transportation systems.

Ensuring adequate housing and basic services.

Promoting energy efficiency and the use of renewable energy sources.

Preserving green spaces and enhancing biodiversity.

Implementing robust waste management and recycling programs.

Addressing Urban Challenges: Governments should focus on tackling the issues that arise from rapid urbanization such as:

Alleviating traffic congestion through better infrastructure and public transportation.

Reducing pollution and improving air and water quality.

Enhancing public safety and reducing crime rates.

Providing adequate social services and healthcare facilities.

Ensuring access to education and employment opportunities.

RESULTS AND DISCUSSION

Regional development, urbanization, and urbanization problems have become serious global issues today. To effectively address these challenges, local governments, national governments, and international organizations must collaborate and develop sustainable solutions. This way, a more livable future can be built to enhance the quality of life for everyone and protect the environment.

Migration to urban environments will persist until conditions in rural areas improve. Considering the challenges posed by rural development, it seems unlikely that the underlying causes will be addressed in the near future. Therefore, governments and development agencies should prioritize adapting to the challenges of urbanization while striving to mitigate unplanned urbanization.

CONCLUSION

By adopting a holistic approach that includes improving rural areas and managing urban growth, we can create a more balanced and sustainable development model. Collaboration among all levels of government and international organizations is crucial for implementing these strategies effectively. This collective effort will help build a future where all individuals can enjoy a higher quality of life in a healthier environment.

REFERENCES

Ahmed F., Zareen M., Khan M.R., Banu C.P., Haq M.N., Jackson A.A. 1998. Dietary pattern, nutrient intake and growth of adolescent school girls in urban Bangladesh. Public health nutrition. 1(2): 83-92.

Ali J., Varshney D. 2012. Spatial Modelling of Urban Growth and Urban Influence: Approach of Regional Development in Developing Economy (India). Journal of Urban and Regional Analysis 4(2): 129–148.

Alirol E., Getaz L., Stoll B., Chappuis F., Loutan L. 2011. Urbanisation and infectious diseases in a globalised world. The Lancet infectious diseases. 11(2): 131-41.

Fuchs R.J., Brennan E., Lo F.C, Uitto J.I., Chamie J. 1994. Mega-city Growth and the Future: United Nations University Press.

Harpham T., Stephens C. 1991. Urbanization and health in developing countries. World health statistics quarterly Rapport trimestriel de statistiques sanitaires mondiales. 44(2): 62-69.

Kuddus A., Tynan E., Mcbryde E. 2020. Urbanization: a problem for the rich and the poor? Public Health Reviews. 41(1): 1–4.

McMichael A.J. 2000. The urban environment and health in a world of increasing globalization: issues for developing countries. Bulletin of the World Health Organization. 78(9): 1117-26.

Moore M., Gould P., Keary B.S. 2003. Global urbanization and impact on health. International Journal of Hygiene and Environmental Health. 206(4-5): 269-278.

Nour N.N. 2010. Obesity in resource-poor nations. Reviews in obstetrics and gynecology. 3(4): 180-184.

Schaible U.E., Stefan H. 2007. Malnutrition and infection: complex mechanisms and global impacts. PLoS medicine. 4(5): 115.

Tomkins A., Watson F. 1989. Malnutrition and infection: a review. United Nations Administrative Committee on Coordination/Subcommittee on Nutrition. Nutrition Policy Discussion Paper. 5: 1-107.

United Nations. 2019. World urbanization prospects. New York.

Wei H., Nian M, Li L. 2023. Studies on Urbanization and Regional Development Strategies and Policies. In F. Xie, F. Cai, X. Li (Eds.) The New Journey of China's Economic and Social Development (pp. 459–498). Springer Nature Singapore.

World Health Organization. 2000. Obesity: preventing and managing the global epidemic: World Health Organization.

World Health Organization. 2006. Obesity and Overweight. World Health Organization, Geneva (Fact sheet no 311).

World Health Organization. 2016. World Health Organization releases country estimates on air pollution exposure and health impact. Geneva: World Health Organization.

WATER RESOURCES FOR IRRIGATION FACING CLIMATE CHANGE IN A SEMI-ARID ENVIRONMENT :CASE OF TEBESSA - ALGERIA

Sedrati Nassima[1], Benmerabet Nadia[1]

1 Water Resources and Sustainable Development Laboratory. Earth Sciences Faculty, Geology Department Badji Mokhtar Annaba University 12P.O.Box,23000Annaba .Algeria
Corresponding author e-mail: nassimasedrati@yahoo.fr

ABSTRACT

In Algeria, several estimates indicate a decrease in rainfall ranging from 20 to 36% since the 1970s. The Tébessa region (Southeast Algeria) is part of the North Aurès region, in the Medjerda Mellègue watershed, with a semi-arid climate where precipitation is less than 400mm/year. Over the past twenty years, the region has experienced a very clear climate change that has led to significant impacts such as a decrease in river runoff and a decline in groundwater levels. This change was characterized by a hydro-pluviometric deficit evaluated at 30%, which had repercussions on all socio-economic activities in this region, especially on its agricultural development. Groundwater constitutes the main source of irrigation water for the plain. This study aims to assess the influence of climate change on groundwater quality. This evaluation was approached by the combined study of the evolution of two climatic parameters (precipitation and temperature) during the period (1986-2020), groundwater levels, and the physico-chemical parameters of groundwater. Thematic maps were developed using Geographic Information System (GIS) to identify the chemical facies of the waters, their quality, and their suitability for irrigation purposes. The results obtained showed that according to the sodium adsorption ratio and percentage sodium, more than eighty percent of the samples are suitable for irrigation. By exploring this data, we can gain a comprehensive understanding of the challenges and opportunities associated with water resources in Tebessa within the context of climate change.

Keywords: Climate change; Quality; Groundwater; Irrigation; GIS

INTRODUCTION

The quality of the water used for irrigation is an essential parameter for crop yield, maintaining soil productivity and protecting the environment. Thus, the physical and chemical properties of the soil, such as its structure (stability of aggregates) and its permeability, are very sensitive to the type of potentially exchangeable ions present in irrigation water.

The study region is one of the regions most vulnerable to climate variability (Laborde, 1993 and Meddi, 2009 - Boudjadja et al, 2003 - Ghenim et al., 2010 and 2013). Major factors on the availability, quality and quantity of water precisely for agriculture and on the productivity of crops over the coming decades.

Groundwater is the main source of irrigation water on the Tebessa Plain. The objective of our work is to assess the quality of groundwater and its suitability for irrigation in the face of climate change. The study of water chemism aims to identify the chemical facies of water, their quality, their origin and their suitability for irrigation

.

MATERIAL AND METHOD

The Morsott region is part of the North Auresian domain, of the Medjerda Mellègue watershed (figure 1). It is limited to the north by El aouinet, Bukhara and El meridj, to the south by Bir Dhab and Boulhaf Dyr, to the east by Ain Zerga, and to the west by Meskiana. The climate is characterized by the alternation of a hot and dry season and a cold and humid season. The hydrographic network is well developed and represented with small temporary watercourses with wide, well-developed valleys flowing into the Oued Chabro which flows upstream of the Oued Mellegue precisely to the south of the commune of El Aouinet.

The study region is part of the North Auresian domain, of the Medjerda Mellègue watershed, is characterized by a semi-arid climate where precipitation is less than 400mm / year.). It has experienced a decrease in rainfall, a decrease in the runoff of rivers and the drop in piezometric levels of groundwater reserves (UNDP. 2009), and for the last 30 years the region subject to a rainfall deficit estimated at 25-30% (FAO 2008) .This rainfall deficit is reflected on all socio-economic activities in this region, especially on its agricultural development.

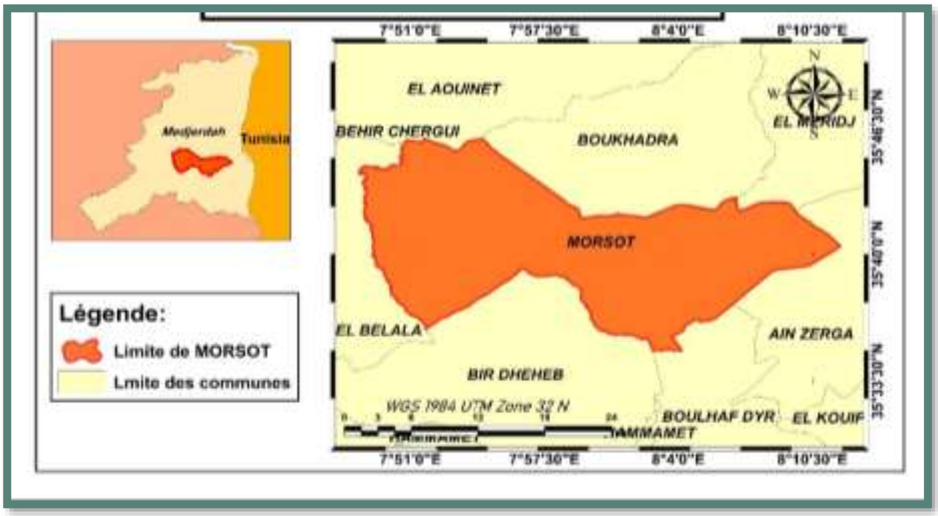


Figure 1. Geographical position of study Area

We carried out the collection of water samples (figure 2) during the month of March. The qualitative study of groundwater is based on the analysis of physicochemical parameters: pH, T °C, electrical conductivities, TDS, Chloride, Nitrate, Sulfate, Sodium, Potassium, Calcium and Magnesium.

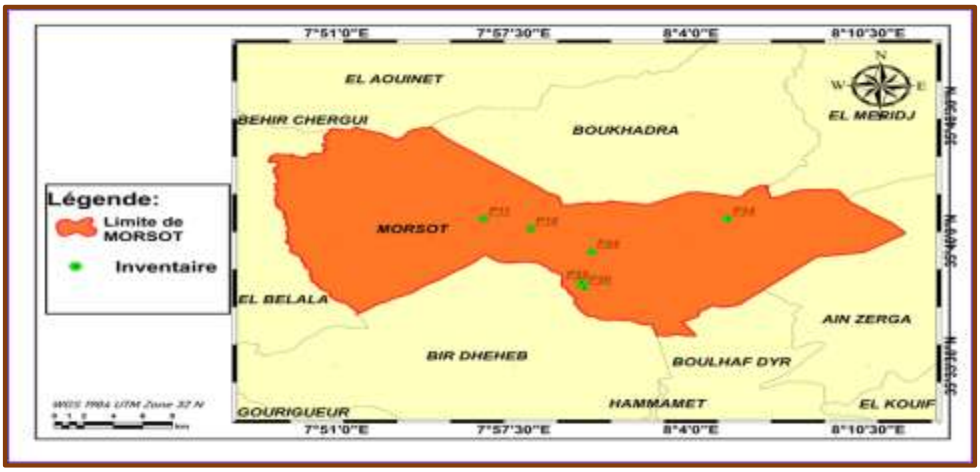


Figure 2. inventory map of sampling

RESULTS AND DISCUSSION

Climate analysis

The observation of the graph (Fig.3) shows an almost cyclical variation of deficit episodes and surplus episodes. The average annual water blade is equal to 362.23 mm. The period from (2000-2001 to 2011-2012), is characterized by a surplus. On the other hand, the driest years are 1993, 2000, 2013 -2014 to 2015-2016

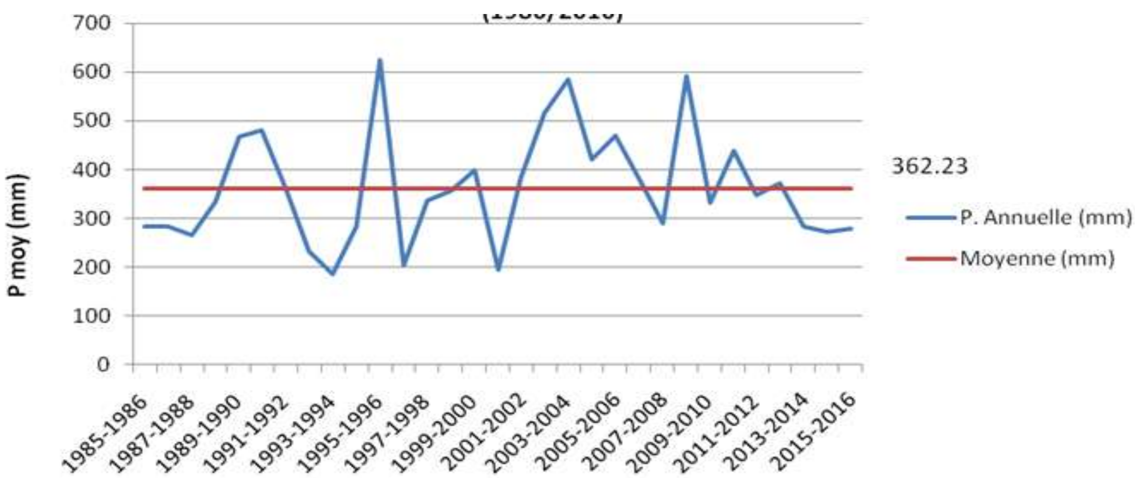


Figure 3 variation of precipitation in study area

The annual mean temperatures vary between 13.5 °C (2008-2009) in the coldest year and 19.08 °C (2000-2001) in the warmest year. The rest varies between 14.5 and 17.3 °C, the mean is 16.75 °C and a standard deviation is 2.79. (Fig.4)

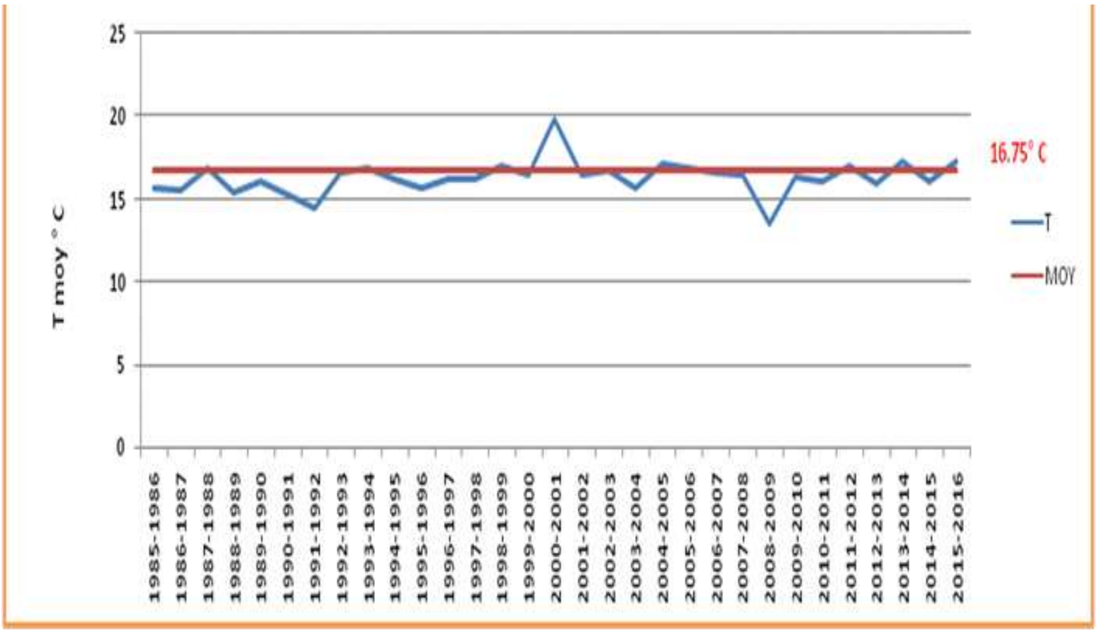


Figure 4 Average annual temperatures of the Tébessa

Physicochemical parameters

Water samples are characterized by a basic pH that varies between 7.80 and 9. The water temperature varies between 15.22 °C and 20.46 °C TDS (total dissolved salts): Very high values vary between 1200 mg/l and 1.728 x104 mg/l An increase in the values of Electrical conductivity varies between 1700 µS/cm and 3000 µS/cm (Fig. 5)

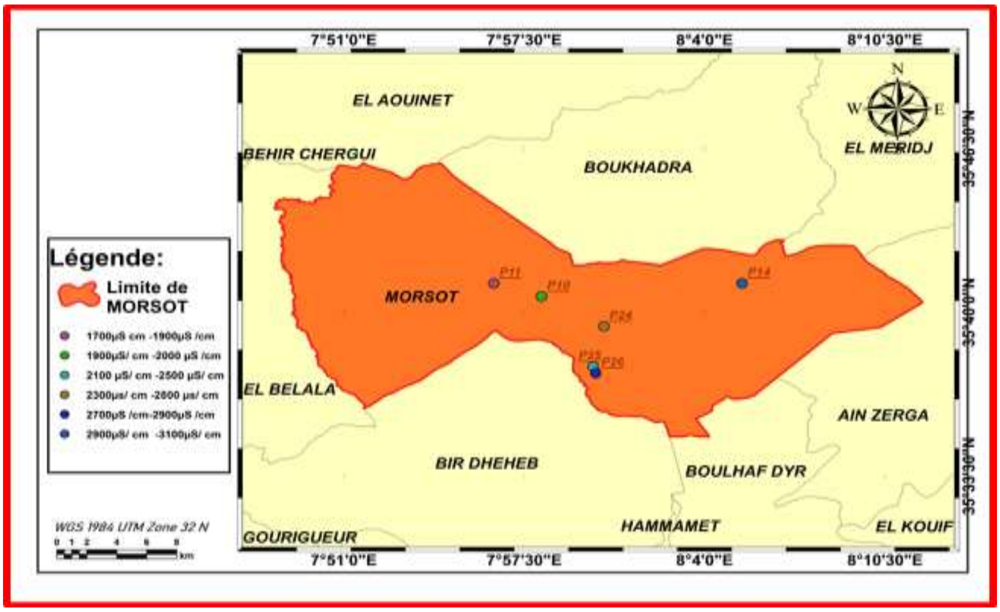


Figure.5.Distribution of electrical conductivity values of samples

Variation of anion and cation contents

HCO3- values ranged between [50mg/l - 80 mg/l]

$SO4^{-2}$ contents ranged between [60 mg/l – 120 mg/l]
Cl^{-} values ranged between [60 mg/l - 160mg/l]
Ca^{+2} values ranged between [02 mg/l - 30 mg/l]
Mg^{+2} contents ranged between [2 mg/l - 160 mg/l]
Na^{+} values ranged between [2 mg/l - 30 mg/l]
K^{+} values ranged between [1.5mg/l - 161 mg/l]

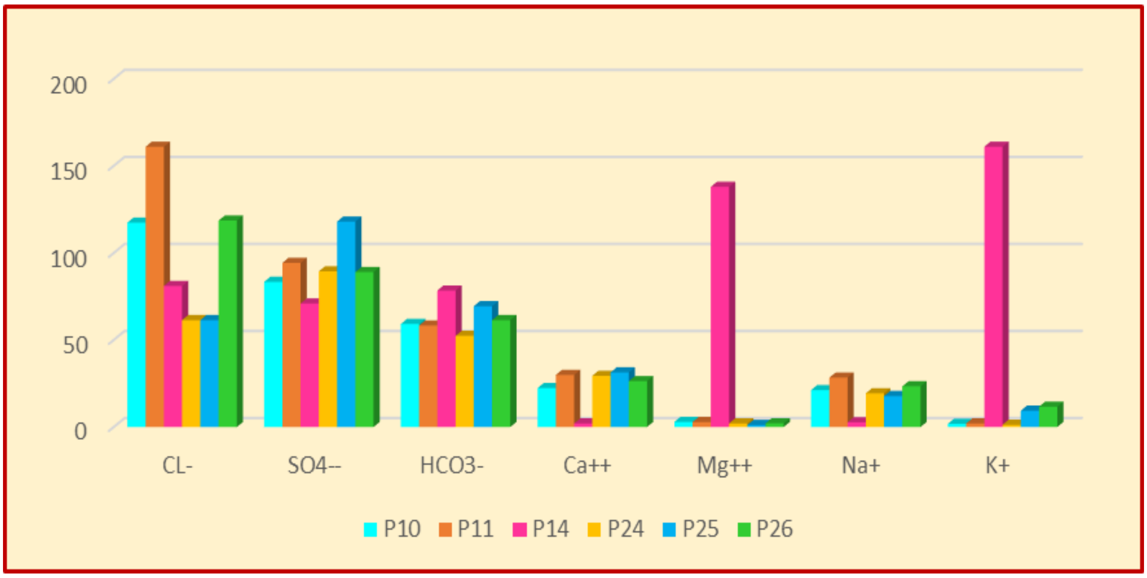


Figure.6. Variation of the contents (Ca+2, Mg+2, Na+, K+) and (Hco3-, Cl-, So4-2) of groundwater

Chemical facies of waters

The samples are distributed between three facies: calcium chloride and calcium sulfate and magnesium chloride Fig.7,

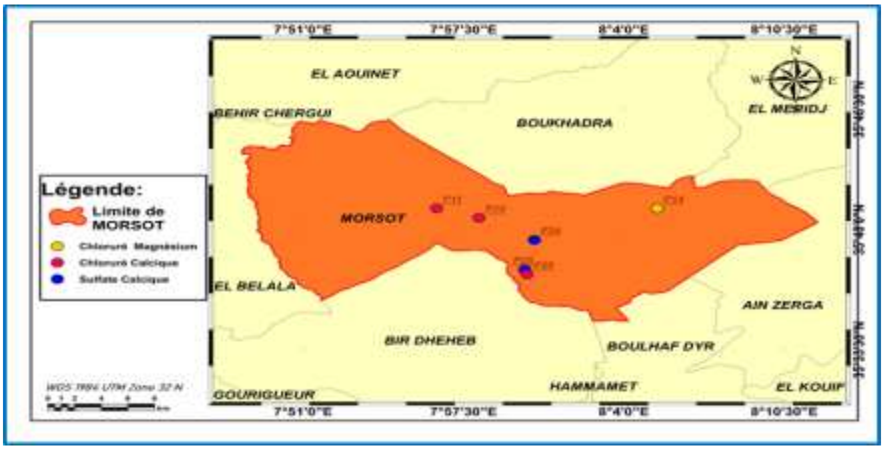


Figure.7. Distribution of chemical facies of the samples

After having plotted all the water points on the RICHARDS diagram (Fig.8) we obtain the following classes: Class C3S1: designates the waters admissible for the irrigation of salt-tolerant crops, on well-drained soils or with good permeability whose salinity must be controlled. Class C4S1: indicates the mediocre waters highly mineralized, likely to be suitable for the irrigation of certain species well tolerant to salts and on well-drained and leached soils.

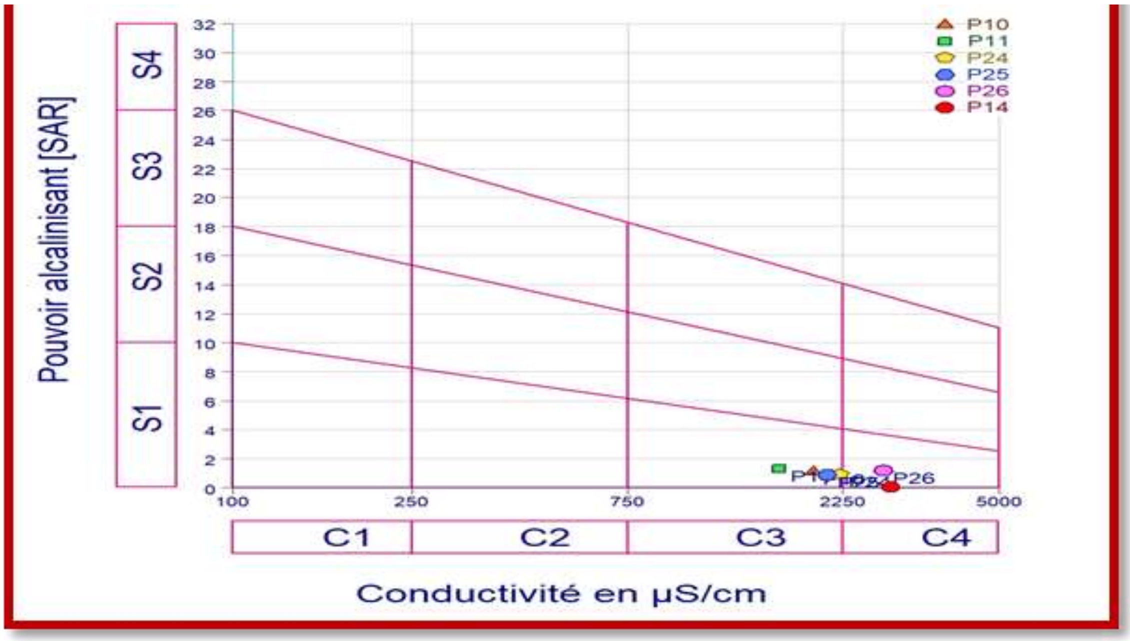


Figure 8. Representation on the RICHARDS diagram) of the waters “March 2021”.

The richness in elements Na^+, Cl^-, Ca^{2+} and SO_4^{--} is linked to the dissolution of minerals likely to form by evaporation of waters loaded with salts and by dissolution of evaporite formations. On the other hand, the elements Ca^{2+}, Mg^{2+} and HCO_3^- are linked to the dissolution of carbonates

CONCLUSIONS

The results obtained showed that the waters are characterized by very high values of total dissolved salts varying between 1200 mg / l and 1.728 x104 mg / l. The values of the electrical conductivity are generally high, varying between 1630 µS / cm and 3100 µS / cm, the pH of the samples is basic which oscillates between 7.80 and 9. The water temperature vary between 15.22 ° C and 20.46 ° C. The samples are distributed between three facies: calcium chloride and calcium sulphate and magnesium chloride. The groundwater in the region is moderately to highly mineralized, which poses a risk of soil damage. This mineralization would come from the dissolution-precipitation -of the aquiferous rock, in particular gypsiferous marls and evaporites.

REFERENCES

Fefdi, Ch. Hydrochemical and Isotopic Contribution in the Characterization of the Mechanisms of Acquisition of Groundwater Salinity in the Morsott El Aouinet Aquifer Complex. Doctorate of science thesis. Badji Mokhtar Annaba University.2008, p 190.

Vila, J.M. The Alpine Chain of Eastern Algeria and the Algerian-Tunisian Borders. Doctorate thesis, Pierre and Marie Curie University, Paris VI.1980.

Talhaoui, A. ; El Hmaidi, A. ; Jaddi, H. ; Ousmana .Calculation of the Water Quality Index (WQI) for the Evaluation of the Physico-Chemical Quality of the Surface Waters of Wadi Moulouya (NE, Morocco). European Scientific Journal. 2020, 16, 1857 – 7881.

Richards, L. Diagnosis and Improvement of Saline and Alkali Soils. In United States Salinity Laboratory ;(vol.60, Agriculture Handbook) ; US Department of Agriculture : Washington, DC, USA, 1954 ; p. 160.

Wilcox, L.V. The Quality of Water for Irrigation Use. US Dep. Agric. Technol. Bull. 1948, 40, 962.hybrids by chlorophyll fluorescence kinetics. Brazilian Journal of Botany. 7(25): 1-12.

AN APPLICATION OF HEAVY METAL POLLUTION INDEX AND HEAVY METAL EVALUATION INDEX TO EVALUATE THE WATER QUALITY OF ATIKHISAR DAM LAKE (ÇANAKKALE, TÜRKIYE)

Cem Tokatlı

Trakya University, Evrenos Gazi Campus, İpsala Vocational School, Department of Laboratory Technology, Edirne / Türkiye
**Corresponding Author: cemtokatli@trakya.edu.tr*

ABSTRACT

Environmental pollution is a significant global problem today and the concentrations of potentially toxic elements (PTEs) in especially freshwater habitats are rising day by day. Dam Lakes are artificial stagnant water bodies built for various purposes such as irrigation and drinking water supply and flood protection. However, over the years, they can become unusable as a result of various factors such as pollution and sedimentation. The Atikhisar Dam Lake that is located in the south-west part of the Marmara Region has a great importance for the local people and for the region. In this research, concentrations of 9 PTEs including chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb), manganese (Mn) and boron (B) were investigated in water of Atikhisar Dam Lake. Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI) were used to evaluate the water qualities of investigated locations in terms of PTEs contamination. According to the applied heavy metal risk assessment indices, water of Atikhisar Dam Lake was recorded as "Low heavy metal contamination (HPI < 100)" in terms of applied HPI and "Low contamination (HEI < 10)" in terms of HEI.

Keywords: Atikhisar Dam Lake, Heavy Metal Pollution Index, Heavy Metal Evaluation Index

INTRODUCTION

Atikhisar Dam Lake is located in the Çanakkale Province of Türkiye and it meets the drinking water needs of the region. Therefore, the reservoir has a very critical impact on the health of many people living in the Çanakkale Province (Anonymous, 2020). The reservoir has a capacity of approximately 53 million m³ and it was built on the Çanakkale Stream in 1975 by State Hydraulic Works (DSİ). The body volume of the reservoir is 1.990.000 m³ and its height from the riverbed is 43 meters. The volume of the reservoir is about 40 hm³ and the reservoir area is about 3.30 km² at the normal water level (Anonymous, 2020; http://suyonetimiormansu.gov.tr).

Toxic metal risk assessment indices are used to evaluate the risk of toxic metal contamination in water and many risk assessment indices are developed to evaluate the synergistic effects of toxic metals. Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI) are 2 of the most significant water quality risk assessment indices used commonly all over the world (Tokatlı et al., 2021; Varol and Tokatlı, 2022; Haque et al., 2022; Jannat et al., 2022; Mutlu et al., 2023; Mia et al., 2023; Tokatlı et al., 2023; 2024; Yüksel et al., 2024). The aim of this research was to evaluate the water quality of Atikhisar Dam Lake in terms of potentially toxic elements (PTEs) by using Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI).

MATERIAL AND METHOD

Study Area and Collection of Samples

In the present study, levels of PTEs in water of Atikhisar Dam Lake were determined and the data were evaluated by using HPI and HEI. Surface water samples were collected from 3 stations (AH 1 – AH3; input of dam, mid of dam and output of dam) selected on the Atikhisar Dam Lake in rainy (winter) season of 2022 (Figure 1).

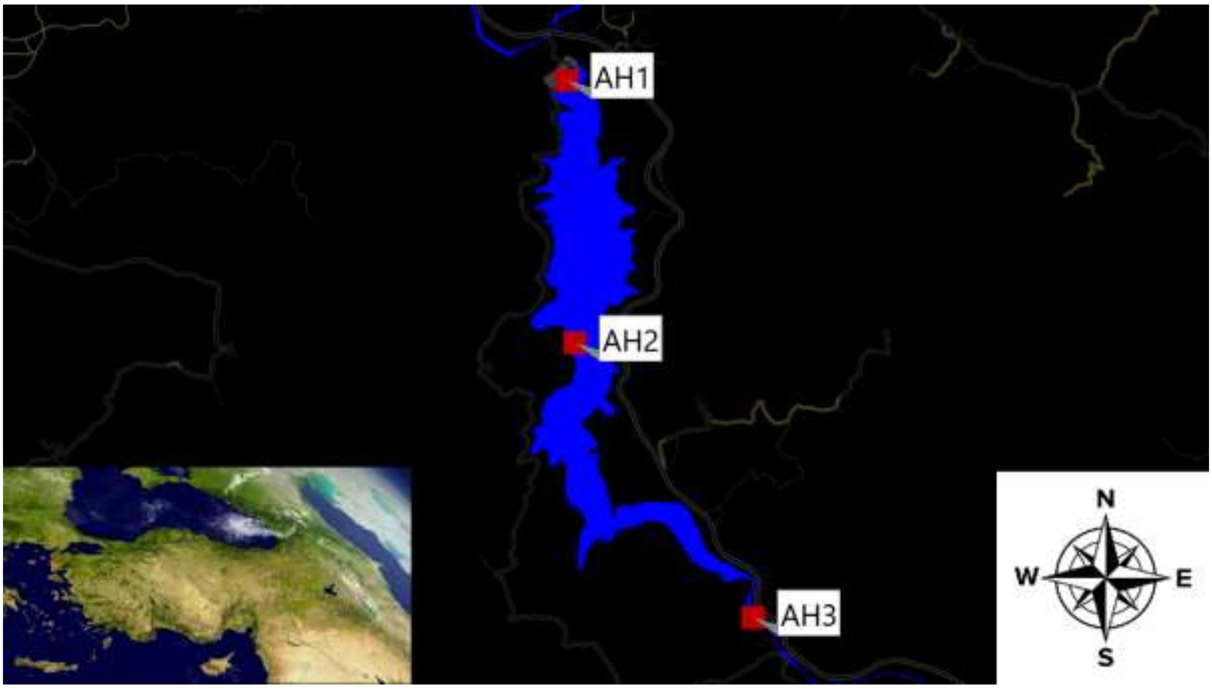


Figure 1. Study area and selected stations

Chemical Analysis

For determination of chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb), manganese (Mn) and boron (B) concentrations in water of Atikhisar Dam Lake, samples of one liter were adjusted to pH 2 by adding 2 ml of HNO_3 into each. Afterwards, all the samples were filtered (cellulose nitrate, 0.45 µm) in such a way as to make their volumes to 50 ml with ultra-pure water. The element levels in water samples were determined by using the "Agilent 7700 xx" branded Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) device in Trakya University Technology Research and Development Application and Research Center (TÜTAGEM). The center has an international accreditation certificate within the scope of TS EN / ISO IEC 17025 issued by TÜRKAK (representative of the World Accreditation Authority in Turkey). The element analyses were recorded as means triplicate measurements (APHA, 1992; EPA, 2001).

Calculation of Risk Assessment Indices

Heavy Metal Pollution Index (HPI) (formulas 1 and 2) (Mohan et al., 1996) and Heavy Metal Evaluation Index (HEI) (formula 3) (Edet and Offiong, 2002) are being calculated according to the following formulas:

$$HPI = \frac{\sum_{i=1}^{n} W_i Q_i}{\sum_{i=1}^{n} W_i} \qquad (1)$$

$$Q_i = \sum_{i=1}^{n} \frac{M_i}{S_i} \times 100 \qquad (2)$$

$$HEI = \sum_{i=1}^{n} \frac{H_C}{H_{MAC}} \qquad (3)$$

“Qi” is the sub – index of the toxic element, “Wi” is the unit weight of the ith parameter, “Mi” is the monitored values of toxic metals, “Si” is the standard values of the parameter and n is the number of parameters considered (WHO, 2011). Water quality ratings for applied HPI are given in Table 1.

"Hc" is value observed for each parameter and "Hmac" indicates the value of maximum admissible concentration (MAC) for each parameter (WHO, 2011). Water quality ratings for applied HEI are given in Table 1.

Table 1. Water quality ratings for indices

Value	Rating of Water Quality	Usage Possibilities
Heavy metal pollution index (HPI)		
< 100	Low heavy metal contamination	Suitable
> 100	High heavy metal contamination	Not suitable
Heavy Metal Evaluation Index (HEI)		
< 10	Low contamination	Suitable
10 – 20	Medium contamination	Not suitable
> 20	High contamination	Not suitable

RESULTS AND DISCUSSION

According to the of applied heavy metal risk assessment indices, water of Atikhisar Dam Lake was recorded as "Low heavy metal contamination (HPI < 100)" in terms of applied HPI and "Low contamination (HEI < 10)" in terms of HEI (Figure 2). The monomial risk ranking of toxic metals were found as in terms of HPI: As > Pb > Mn > Cd > Ni > Cr > Cu > Zn > B; while the risks in terms of HPI were as follows: As > Mn > Pb > Ni > Cd > Cr > Cu > Zn > B (Figure 3). It was also determined that although the water quality of Atikhisar Dam Lake increases from input stations to output station, water of the whole reservoir found as "Suitable for consumption and use", in general.

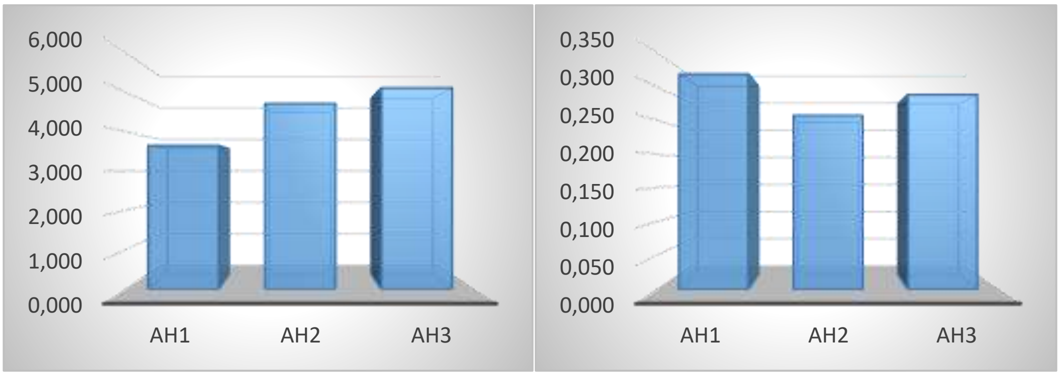


Figure 2. Results of applied HPI (left) and HEI (right)

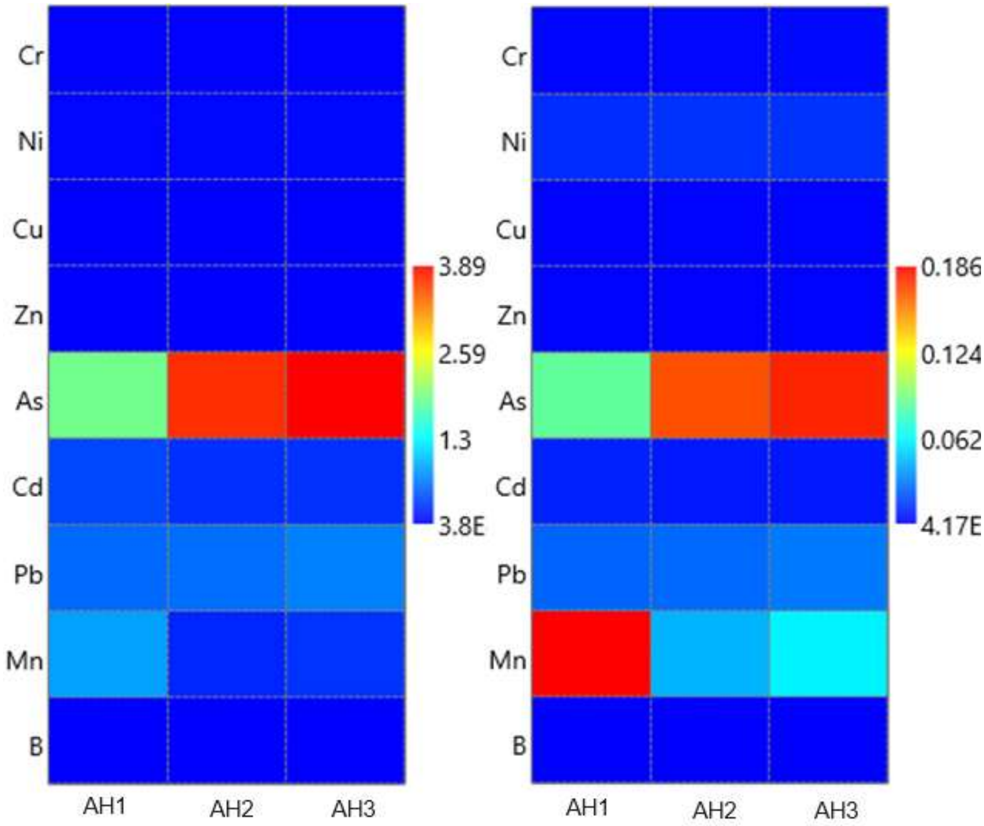


Figure 3. Monomial results of applied HPI (left) and HEI (right)

CONCLUSIONS

In this research, water quality of Atikhisar Dam Lake located in the Çanakkale Province of Türkiye was evaluated in terms of PTEs contamination by using HPI and HEI. According to the results of applied toxic metal risk assessment indices, Atikhisar Dam Lake was found as "Low heavy metal contamination" in terms of applied HPI and "Low contamination" in terms of HEI. The data of the present research also reflects the importance, applicability and necessity of the use of different toxic metal risk assessment indices together on evaluation of surface water ecosystems.

ACKNOWLEDGEMENTS

This research was supported by Trakya University Scientific Research Projects (project no: 2022/63).

REFERENCES

Anonymous, 2020. 2020 Environmental status report of Çanakkale Province. Republic of Türkiye, Çanakkale Governorship, Provincial Directorate of Environment and Urban Management.

APHA (American Public Health Association). 1992. Standard methods for the examination of water and wastewater. In A.E. Greenberg, A.E., Clesceri, L.S. and Eato, A.D. (eds.) American Public Health Association, 18th ed., Washington, U.S.A.

Edet, A.E., Offiong, O.E., 2002. Evaluation of Water Quality Pollution Indices for Heavy Metal Contamination Monitoring. A Study Case from Akpabuyo-Odukpani Area, Lower Cross River Basin (Southeastern Nigeria). GeoJournal, 57, 295–304.

Environmental Protection Agency (EPA) METHOD 200.7. 2001. Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry.

Haque, J., Siddique, A. B., İslam, S., Ali, M. M., Tokatlı, C., İslam, A., Pal, S. C., İdris, A. M., Malafaia, G., İslam, A. R. T., 2023. Effects of covid-19 era on a subtropical river basin in bangladesh: heavy metal(loid)s distribution, sources and probable human health risks. Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2022.159383.

http://suyonetimiormansu.gov.tr

Jannat, J. N., İslam, K. M. S., İslam, H. M. T., İslam, M. S., Khan, R., Bakar, S. M. A., Varol, M., Tokatlı, C., Chandra, P. S., İslam, A., İdris, A. M., Malafaia, G., Islam, A. R. T., 2022. Hydrochemical assessment of fluoride and nitrate in groundwater from east and west coasts of Bangladesh and India. Journal of Cleaner Production, https://doi.org/10.1016/j.jclepro.2022.133675.

Mia, Y., İslam, A. R. T., Jannat, J. N., Jion, M. M. F., Sarker, A., Tokatlı, C., Siddique, A. B., İbrahim, S. M., Senapathi, V., 2023. Identifying factors affecting irrigation metrics in the Haor Basin using integrated Shannon's Entropy, fuzzy logic and automatic linear model. Environmental Research, 226: 115688.

Mohan, S.V., Nithila, P., Reddy, S.J. 1996. Estimation of Heavy Metal in Drinking Water and Development of Heavy Metal Pollution Index. Journal of Environmental Science and Health, Part A, 31 (2), 283–289.

Mutlu, E., Tokatlı, C., Islam, A. R. T., İslam, S., Muhammad, S., 2023. Water quality assessment of Şehriban Stream (Kastamonu, Türkiye) from a multi-statistical perspective. International Journal of Environmental Analytical Chemistry, https://doi.org/10.1080/03067319.2023.2197114.

Tokatlı, C., Mutlu, E., Arslan, N., 2021. Assessment of the potentially toxic element contamination in water of Şehriban Stream (Black Sea Region, Turkey) by using statistical and ecological indicators. Water Environmnet Research, 93: 2060–2071.

Tokatlı, C., Onur, Ş. G., Dindar, M. B., Malafaia, G., Islam, A. R. T., Muhammad, S., 2023. Spatial-temporal variability and probabilistic health risk assessment of fluoride from lentic ecosystem, Türkiye. International Journal of Environmental Analytical Chemistry, https://doi.org/10.1080/03067319.2023.2198645.

Tokatlı, C., Mutlu, E., Ustaoğlu, F., Islam, A. R. T., Muhammad, S., 2024. Spatiotemporal Variations, Health Risk Assessment, and Source Changes of Potentially Toxic Elements in Potamic Water of the Anday Stream Basin (Türkiye), Black Sea Region. Environmental Monitoring and Assessment, 196: 420.

Varol, M., Tokatlı, C., 2022. Seasonal variations of toxic metal(loid)s in groundwater collected from an intensive agricultural area in northwestern turkey and associated health risk assessment. Environmental Research, 204, Part A: 111922.

World Health Organization (WHO), 2011. Guidelines for Drinking-water Quality. World Health Organization Library Cataloguing-in-Publication Data, NLM classification: WA 675.

Yüksel, B., Ustaoglu, F., Aydın, H., Tokatlı, C., Topaldemir, H., Islam, S., Muhammad, S., 2024. Appraisal of Metallic Accumulation in the Surface Sediment of a Fish Breeding Dam in Türkiye: A Stochastical Approach to Ecotoxicological Risk Assessment. Marine Pollution Bulletin, 203: 116488.

GENETIC DIVERGENCE OF SOME WEEDY BRASSICACEAE SPECIES INFERRED FROM rDNA ITS LOCUS

Erdal ATEŞ [1], Zeynep TOPRAK[2]*

[1] *Diyarbakir Plant Protection Research Institute, Department of Herbology, Diyarbakır, Turkey*

[2] *Department of Molecular Biology and Genetics, Faculty of Sciences, University of Dicle, Diyarbakir, Turkey*

Corresponding author: *erdal.ates@tarimorman.gov.tr*

ABSTRACT

Brassicaceae is a large, and economically important plant family with a broad distribution. Some weedy species of the Brassicaceae family have critical impact on the global agriculture. In particular, those occupy cultivated areas can cause serious yield losses depending on the species and its density in the corresponding area. Identification of the weedy species and their phylogenetic relationships can help with sustainable controlling methods and therefore improve the agricultural production. Here, we attempted to identify, and also understand genetic relationships of the common Brassicaceae species occur in the wheat fields investigated from several provinces of Turkey, based on the nuclear ribosomal DNA (rDNA) sequence data. The study material was obtained from the seeds of weedy Brassicaceae species collected from their wild populations and subsequently germinated in greenhouse. Our results displayed presence of *Sinapis arvensis*, *S. alba*, *Chorispora purpurascens*, *Sisymbrium altissimum*, *Diplotaxis tenuifolia*, *Brassica elongate*, *Lepidium draba* and *Capsella bursa-pastoris* as the common Brassicaceae members found in the studied areas. Several of these species (e.g., *S. arvensis*, *S. alba*, *C. bursa*) were observed with considerable genetic divergence.

Keywords: agriculture, brassicaceae, rDNA, weed, wheat

INTRODUCTION

Brassicaceae is a large plant family exist across a wide range of habitats in the world.
The family includes 4000 species that are mainly adapted to the temperate and tropical regions of the world. While some of these species are economically important plants like cabbage, oilseed rape, mustard, and broccoli cultivated for food and industrial purposes etc., some others are invasive plants such as wild mustard, garlic mustard etc. (Appel and Al–Shehbaz 2003; Warwick et al. 2009).

Weedy Brassicaceae species can be detrimental to agricultural production. Previous studies (Ateş and Üremiş 2022) have reported the losses of the complete yield resulted from the weedy species took advantages of the rainy seasons. Besides hosting diseases and pests, since they compete for the nutrients, water, and light, weeds can considerably reduce the amount and the quality of the grown crops (Plaza et al. 2022). Weed invasions, also have a large negative impact on the wheat production, the cereal of a great importance to the global agriculture. For instance, weed seeds commix with the raw grains at harvest can significantly lower the quality of the composition, colour, flavour, and smell of the durum wheat (Ateş and Üremiş 2018). Moreover, such seeds can be toxic for the consumers including livestock (Keywanloo et al. 2021).

Controlling of the weedy species is usually performed by using herbicides function differently, a process known with limited success despite the economic and environmental costs. Improper applications of the herbicides, one of the common reasons of (Sevim et al. 2023) the poor success, frequently resulted from the incorrect identification of the weeds. Unfortunately, many weedy Brassicaceae species are confused by non–specialists due to their morphological aspects such as yellow flowers, and therefore treated with chemicals of no use. Furthermore, several different weedy species may co-exist in the cultivation land, a case requires more careful actions as each species needs to be evaluated separately. Briefly, the effective weed controlling can be achieved primarily by accurate characterization of the weeds, and that includes understanding of the weed species and their origins, how they adapt to a particular environment, and the genetic basis of their adaptations, and also the level of the preserved genetic variations (Sakai et al. 2001). This knowledge can help in finding more efficient ways of coping with the weeds.

The work presented here is a preliminary study focuses on the identification of the most common weedy Brassicaceae species occupy wheat growing fields in various regions of Turkey. By using sequence data of nuclear ribosomal DNA (rDNA) ITS locus, one of the most widely used markers in phylogenetic studies of the family Brassicaceae (German et al. 2009, Warwick et al. 2009), we have attempted to firstly identify the collected weeds, and then understand their genetic relationships which may provide insights into their controlling methods.

MATERIALS AND METHODS

Plant material was collected during 2020–2021 from wheat growing fields distributed to 10 provinces in the east, south–eastern, northern and western Turkey regions. In each province, three separate fields were visited, and approximatley15 Brassicaceae samples had reached to seed maturity were collected from each location. Collected samples were initially morphologically evaluated based on the literature (e.g., Kloos 1927; Ibérica 1993; González 2014) as well as the virtual herbaria, and grouped accordingly. To get the fresh material of the sampled plants, seeds from selected individuals were treated with 50% NaOH solution for 10 minutes in priori and germinated in an incubator at 20°C (Ateş et al. 2017). Germinated seeds were moved to the pots containing a mixture of peat, soil, and sand (1:1:1). The samples were kept in greenhouse (24 ºC ± 2 and 40% humidity) until they sufficiently grown. DNA isolation was made from the fresh leaves processed with liquid nitrogen (LN2) using the DNeasy Plant Mini Kit (Qiagen, Germany). Amplification of the rDNA ITS locus was performed via Vivantis–DNA–amplification kit (Vivantis, Lithunia) under the manufacturer's instructions. PCR and sequencing reactions were carried out with the universal primers of

ITS4 and ITS5. All amplified products were checked on an agarose gel, and the samples with proper bands were sent to BM Labosis (Ankara, Turkey) for sequencing.
Chromatograms were visualized and edited via UGENE–38.1. under the default settings. The final sequences were blasted (Johnson et al. 2008) against standard databases using "blastn" algorithm provided by National Center for Biotechnology Information (NCBI). The sequences hit to the queries with 95% or above similarity are considered for the determination of the collected samples in addition to the morphological evidence. Identified sequences were subjected to multiple sequence alignment using MAFFT as implemented in Geneious Prime 2022.1.1 (Biomatters, USA), and afterwards, manually adjusted regarding the improper positions. Genetic relationships of aligned sequences were estimated via PhyML, a Maximum Likelihood (ML) phylogenetic approach, available in https://ngphylogeny.fr (Dereeper et al. 2008, Lemoine et al. 2019). The estimated ML tree of the ITS sequences was illustrated with FigTree v1.4.4 (Rambaut 2018).

RESULTS AND DISCUSSION

The morphological examination and data from GenBank supported for the presence of a minimum10 distinct Brassicaceae species (excluding those we were not able to identify). Our results showed the occurrence of *Sinapis arvensis* L., Sinapis. *alba* L., *Chorispora purpurascens* (Banks & Sol.) Eig, *Sisymbrium altissimum* L., *Diplotaxis tenuifolia* (L.) DC, *Lepidium draba* L., Capsella bursa-pastoris (L.) Medik, and *Brassica elongata* Ehrh. as the common Brassicaceae members found in the studied wheat fields. Despite the effort, several *Sisymbrium* samples could not be determined at species level since their similarity to sequences listed by standard databases stayed below the thresholds value (%95) applied to the blast–search, neither the morphological features provided a clear sign. In order to be resolved precisely, such samples are required either a more detailed morphological characterization or sequencing of other loci except than ITS. In total, molecular data matrix was constructed with 15 sequences representing all studied samples. The alignment length of these 15 sequences is observed as 796 nucleotide characters long. Phylogenetic relationships of the identified samples are displayed in Figure. 1. Based on the phylogenetic analysis, the studied areas are occupied by fairly divergent weeds.

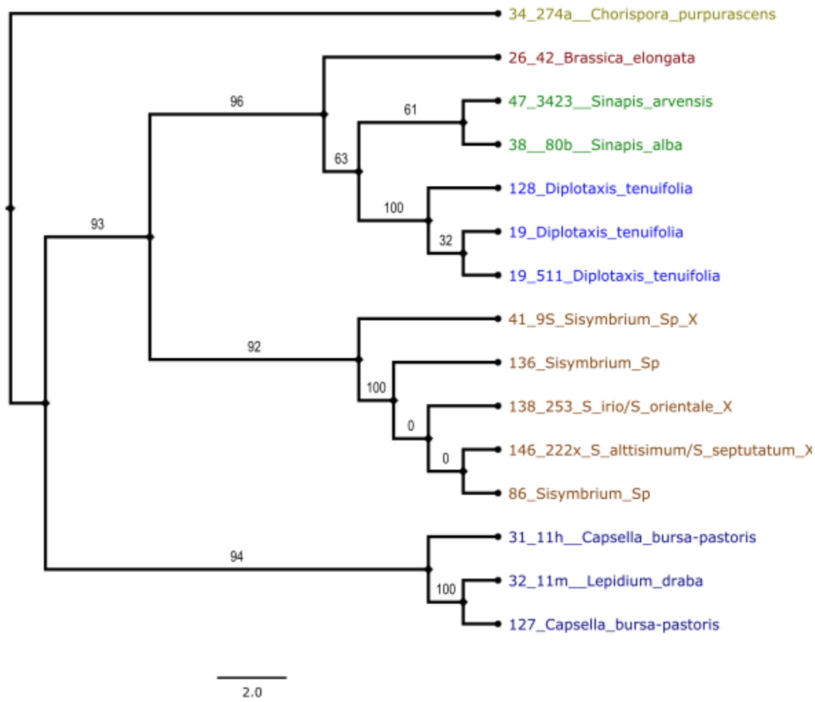


FIGURE 1. Phylogenetic relationships of the Brassicaceae samples based on ITS locus, inferred via Maximum Likelihood method. Bootstrap values (Bs) are placed above the corresponding branch. "X" appears at the end of the tip's name denotes the uncertainty associated with the diagnosis of the sample. Scale bar reflects the number of substitutions per site. The tree is rooted from midpoint for visualization, and branches are shown as proportional.

ML gene tree placed the studied samples in to four well resolved major clades that correspond to the defined genera. Genera *Brassica*, *Sinapis*, *Diplotaxis* and *Sisymbrium* are positioned as closer compared to the genera *Capsella*, and *Lepidium*. The relationships among the studied genera are largely congruent with the results generated by earlier studies (e. g., Zhao et al., 2010). In agreement with the recent studies (e.g., Inaba and Nishio 2002), *S. alba* and *S. arvensis* are demonstrated as sister species however, without support (Bs: 61). *C. purpurascens* is observed as the species most distantly related to the rest of the samples, as expected from examined morphological aspects.

CONCLUSIONS

Identification of the weed species and their genetic relationships can potentially be important for developing sustainable and effective controlling methods. Our study verifies the common weedy Brassicaceae species occur in the wheat growing fields in Turkey. We showed that, all 10 of the identified weed species have a wide range of morphological and genetic differentiation, co-exist in a single cultivation area. These results may specifically be beneficial for the herbicide selection that should be based on the full weed diversity of the area in question.

REFERENCES

Appel, O., Al–Shehbaz, I.A. 2003. Cruciferae. In: K Kubitzki, ed. The families and genera of vascular plants, vol 5. Berlin, Germany: Springer, 75–174.

Ateş, E., Süer, İ.E., Üremiş, İ. 2017. Germination Biology of Wild Mustard (Sinapis arvensis L.). 3RD ASM International Congress of Agriculture and Environment. 16–18 November 2017, Antalya.

Ateş, E. and Üremiş, İ. 2018. Determination of germination temperatures of Sinapis arvensis L. and Avena sterilis L. International Journal of Agricultural and Natural Sciences, 1(2), 154–159.

Ateş, E. and Üremiş, İ. (2022). Determination of Weed Species and Their Frequency and Density in Wheat Fields in Batman Province, Turkey. Turkish journal of weed sciences, 25(1), 9–19.

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.F., Guindon, S., Lefort ,V., Lescot, M., Claverie, J.M., Gascuel, O. 2008. Phylogeny.fr: robust phylogenetic analysis for the non–specialist. Nucleic Acids Research. Jul 1;36(Web Server issue):W465–9. doi: 10.1093/nar/gkn180. Epub 2008 Apr 19. PMID: 18424797; PMCID: PMC2447785.

German, D.A., Friesen, N., Neuffer, B. *et al*. 2009. Contribution to ITS phylogeny of the Brassicaceae, with special reference to some Asian taxa. Plant Systematics and Evolution, 283, 33–56.

González, J. 2014. Flora silvestre de Hoyo de Manzanares. https://bibdigital.rjb.csic.es/records/item/15934–redirection (6.2.2020)

Guindon, S., Gascuel, O. 2003. PhyML: A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Systematic Biology. 52(5):696–704.

Ibérica, F. 1993. Plantas vasculares de la Península Ibérica e Islas Baleares (Cruciferae–Monotropaceae) vol. IV, Real Jardín Botánico. CSIC. Madrid.

Inaba, R., Nishio, T. 2002. Phylogenetic Analysis of Brassiceae Based on The Nucleotide Sequences of The S–locus Related Gene, SLR1. Theoretical and Applied Genetics, 105(8), 1159–1165.

Johnson, M., Zaretskaya, I., Raytselis,Y., Merezhuk,Y., McGinnis, S., Madden T.L. 2008. NCBI BLAST: a better web interface, *Nucleic Acids Research*, Volume 36, Issue suppl_2, 1 July 2008, Pages W5–W9.

Keywanloo, M., Shahroozian, E., Ahmadi–Hamedani, M., Javaheri–Vayeghan, A. and Emadi–Chashmi, H. 2021. Possible Acute Poisoning by Sinapis arvensis in Sheep: Clinical, Laboratory and Necropsy Findings. Archives of Razi Institute, 76(3), 699.

Lemoine, F., Correia, D., Lefort, V., Doppelt–Azeroual, O., Mareuil, F., Cohen–Boulakia, S., Gascuel, O. 2019. NGPhylogeny.fr: new generation phylogenetic services for non–specialists, Nucleic Acids Research, Volume 47, Issue W1, 02 July 2019, Pages W260–W265.

Kloos, A.W. 1927. Aanwinsten van de Nederlandse Flora in 1923 (1924 en 1925). Nederlandsch kruidkundig archief. Serie 3, 36(1), 75–116.

Plaza, H.E., Bastida, F., Gibson, D.J., Barro, F., Giménez, M.J., Pallavicini, Y., ... and González–Andújar, J. L. 2022. Grain quality as influenced by the structural properties of weed communities in Mediterranean wheat crops. Agronomy, 13(1), 49.

Rambaut, A. 2018. FigTree v1.4.4. Institute of Evolutionary Biology, University of Edinburgh, Edinburgh. http://tree.bio.ed.ac.uk/software/figtree

Sakai, A. K. , Allendorf, F. W. , Holt, J. S. , & Lodge, D. M. 2001. The population biology of invasive species. Annual Review of Plant Biology, 32, 305–332.

Sevim, Ş., Boyacı, M., Gülmez, M., Ateş, E., Karakaş, U.S., Kaya, C., Orak, A.B. 2023. Knowledge Level of Producers in Pesticide Use: the Case of Diyarbakır Province. Eurasian *Journal* of Researches in Social and Economics, 10(1), 247–257.

Warwick, S.I., Sauder, C.A., Mayer, M.S., Al–Shehbaz, I.A. 2009. Phylogenetic relationships in the tribes Schizopetaleae and Thelypodieae (Brassicaceae) based on nuclear ribosomal ITS region and chloroplast *ndh*F DNA sequences. Botany 87:961–985.

Zhao, B., Lei Liu, L., Tan D., Wang, J. 2010. Analysis of phylogenetic relationships of Brassicaceae species based on Chs sequences,Biochemical Systematics and Ecology, Volume 38, (4):731–739.

DETERMINATION OF THE WEED SEEDS CONTAMINANTS OF THE WHEAT GROWN IN BİNGÖL PROVINCE

Erdal ATEŞ [1], Nihat TURSUN[2], İlhan ÜREMİŞ[3], Zeynep TOPRAK[4]*

[1]*Diyarbakir Plant Protection Research Institute, Department of Herbology, Diyarbakır, Turkey,* orcid: 0000-0002-3708-3407

[2]*Department of Plant Protection, Faculty of Agriculture, Turgut Ozal University, Malatya, Turkey,* orcid: 0002-8765-0326

[3]*Department of Plant Protection, Faculty of Agriculture, Mustafa Kemal University, Hatay, Turkey,* orcid*:0000-0001-5937-9244*

[4]*Department of Molecular Biology and Genetics, Faculty of Sciences, University of Dicle, Diyarbakir, Turkey,* orcid*: 000-0002-2103-4141*

Corresponding author: *erdal.ates@tarimorman.gov.tr*

ABSTRACT

Being one of the top most-produced cereal wheat is a crop of great importance to the global agriculture. Turkey is one of the countries with highest wheat cultivation. The quality and amount of the wheat can be drastically reduced by the weedy species coexist in the growing area. Such species not only compete for nutrients, water, and light of the crop, but also host diseases and pests, which all jointly lower the production. The knowledge of weedy species in priori can prevent mixing of them with harvest if proper strategies of weed control are applied. It may also help with the optimization of harvesting machines, and related post-harvest processes. Advances in these procedures can improve the crop production and lower the labour, and other environmental costs. This study focuses on the determination of weed seeds contaminants of the wheat produced in Bingöl, Turkey. The plant material was obtained from the wheat grains cultivated in 2022. Following the harvest, samples were collected from the wheat stacks representing each wheat growing field. Using a mechanical separation method, the seed material was sorted and categorized based on the morphological observations. The results showed presence of 33 weedy species contaminate the studied wheat stacks. The mixing incidence of all the determined weedy species was found to be 5.8 %, which indicate the poor controlling of the weeds in the wheat cultivated areas in Bingöl.

Keywords: Turkey, Bingöl, harvest, weeds, wheat

INTRODUCTION

Wheat (*Triticum* spp.) is an important crop cultivated across the globe. It has been used as the main energy supply in the alimentation of different cultures throughout history. According to FAO (2024) data, approximately 761 million tons of wheat are produced worldwide. In Turkey, in an area of about 86 million hectares, 20.5 million tons of wheat are produced per year (FAO, 2024). The amount of produced wheat can be limited by a number of biotic and abiotic factors. Among the biotic factors, weeds can lower the yield at a rate of 15-52% (Ateş, 2022). In fact, in rainy seasons, weeds can destroy all the yield by invading the entire wheat field. Besides the problems seen during the active growing period, weeds cause a series of troubles at harvest, and even at post harvesting stages like storage and processing.

Depending on ecological conditions, weed behaviours deviate from those of cultivar's (Buhler, 2003). For instance, some weedy species emerge following the end of chemical controlling period. In this case, such species are picked at the harvest (Karlsson and Milberg, 2007; Ateş and Üremiş, 2018), and their seeds commix to the wheat grains. They can also, disrupt the regulation and efficiency of the harvesting machines (Zimdahl, 2018). Moreover, they may lead to rotting of the stored yield due to their wet content introduced to the dried crop (Oerke, 2006). Further, these undesired contaminants lower the market value of the product (Tursun et al., 2006). Post-harvest refining of the weed seeds, on the other hand, higher the costs and labour. As summarized above, controlling of the weedy species in wheat fields is essential to increase the amount and quality of the product. This can be done either with the cultivation methods, which are related to the planting strategies, and-or via chemical controlling. Chemical controlling is based on the herbicides that are applied in various time schemes such as pre-planting, pre/post-emergence, depending on the mechanisms of the action (HRACGLOBAL, 2024). Thus, at each stage of the production, determination of the type and density of the weeds commix to the product is crucial for improving agricultural production. This study aims to determine the weed seeds and their densities in the wheat sampled from the wheat stacks harvested from Bingöl province in 2022.

MATERIAL AND METHODS

Plant material of the study is composed of wheat seeds and their contaminants harvested in Bingöl province at the end of the growing season of 2022. The material was obtained from the wheat stacks sampled from randomly selected villages. Each visited stack was sampled by drilling 1 kg of wheat from 4 different points (Gökalp and Üremiş, 2015). In total, 4 kg of wheat were taken from each of the wheat stack. The collected material was sifted using mechanical and physical techniques, and grouped accordingly. The categorized weed seeds were then examined under a microscope, and diagnosed based on the related literature. The seeds could not be determined were grouped as others. Each group of weed seeds were counted and weighed. The "occurrence frequency" of the species was calculated according to Baş (2011). The "mixing ratios" was determined as the "ratio of the amount of detected weed seeds" to the "full amount of the sample" (Tursun et al., 2006). The prevalence of the weed seeds was defined regarding the scale given in Pamukoğlu (2011), expressed as following: Very Common (CR): >50.0%, Common (CR): 25.0-49.9%, Significant (CR): 10.0-24.9%, Rare (NR): 9.9%>.

RESULTS AND DISCUSSION

The details of the weed seeds were detected in the wheat harvested from Bingöl province are presented in Table 1. A total of 4810 seed grains were counted from 1 kg of wheat sample. Their total weight was measured as 58.5 g, and the mixing ratio was estimated to be 5.8%. We detected 33 weedy species belonging to the 10 plant families cover 29 genera. Distribution of these species to the families follows as: Apiaceae (3), Asteraceae (3), Brassicaceae (5), Convolvulaceae (2), Dipsacaceae (1), Fabaceae (5), Papaveraceae (1), Poaceae (9), Ranunculaceae (2) and Rubiaceae (2). While 9 of these species are assigned to the "narrow-leaved", 24 of them are placed in the "broad-leaved" weed groups.

Table 1. Details of the contaminants of the wheat harvested from Bingöl province in 2022. The estimates are based on 1 kg of wheat.

Weed species	Family	Weight(g	Mixing rate (%	Amount (Grain)	Frequency*
Adonis sp.	Ranunculaceae	0.178	0.018	47.337	NR
Aegilops sp	Poaceae	0.693	0.069	23.424	ÖR
Aegilops umbellulata Zhuk.	Poaceae	0.224	0.022	2.416	ÖR
Alyssum sp.	Brassicaceae	0.002	0.000	1.613	NR
Avena spp	Poaceae	0.498	0.050	22.873	ÖR
Brassica elongata Ehrh.	Brassicaceae	0.057	0.006	8.621	NR
Bromus tectorum L.	Poaceae	0.150	0.015	22.472	NR
Cardaria draba (L.) Desv.	Brassicaceae	0.005	0.001	5.282	NR
Caucalis daucoides L.	Apiaceae	0.511	0.051	38.700	NR
Centaurea solstitialis L.	Asteraceae	0.195	0.020	7.317	NR
Cephalaria syriaca L.	Dipsacaceae	0.174	0.017	58.140	NR
Convolvulus arvensis L.	Convolvulaceae	1.029	0.103	94.195	YR
Convolvulus spp.	Convolvulaceae	0.037	0.004	9.057	ÖR
Eryngium sp.	Apiaceae	3.578	0.358	29.412	NR
Galium sp.	Rubiaceae	5.012	0.501	1147.011	ÖR
Galium tricornutum Dandy	Rubiaceae	2.521	0.252	259.297	ÇR
Hordeum murinum L.	Poaceae	9.052	0.905	339.249	ÖR
Hordeum spontaneum Koch.	Poaceae	5.295	0.529	246.141	YR
Lolium perenne L.	Poaceae	0.414	0.041	25.069	ÖR
Medicago sativa L.	Fabaceae	0.262	0.026	7.491	NR
Papaver sp.	Papaveraceae	0.148	0.015	41.420	NR
Picnomon acarna (L.) Cass.	Asteraceae	0.220	0.022	21.951	NR
Pisum sativum L.	Fabaceae	0.730	0.073	11.236	NR
Poa bulbosa L.	Poaceae	0.300	0.030	9.363	NR
Ranunculus arvensis L.	Ranunculaceae	6.252	0.625	163.232	ÇR
Secale sp.	Poaceae	15.988	1.599	1787.791	NR
Sinapis arvensis L.	Brassicaceae	0.622	0.062	204.025	YR
Sonchus arvensis L.	Asteraceae	0.004	0.004	0.899	ÖR
Thlaspi arvense L.	Brassicaceae	0.016	0.002	8.065	NR
Trifolium sp.	Fabaceae	0.018	0.002	1.799	NR
Turgenia latifolia (L.) Hoffm.	Apiaceae	0.349	0.035	14.535	NR
Vicia narbonensis L.	Fabaceae	2.817	0.282	107.756	ÖR
Vicia sp.	Fabaceae	0.431	0.043	21.152	YR
Diğerleri		0.433	0.043	22.454	

*Very Common (CR): >50.0%, Common (CR): 25.0-49.9%, Significant (CR): 10.0-24.9%, Rare (NR): 9.9%>

R. arvensis was observed with the highest frequency (0.625%) among the species having a "prevalence ratio" of higher than %25. *H. spontaneum* (0.529%), *G. tricornutum* (0.252%), *C. arvensis* (0.103%), *S. arvensis* (0.062%), and *Vicia sp*. (0.043%) were the ones following *R. arvensis*, respectively.

In their survey conducted in the wheat fields of Bingöl province, Esim and Çoruh (2021) reported presence of 109 weed species which encompass the 33 ones that are recorded in our work. Our findings also overlap with the prevalence, and density estimates reported by Esim and Çoruh (2021).

The contaminants of the wheat produced in Bingöl province are similar to the weed seeds contaminants of the wheat grown in Mardin (Gökalp and Üremiş, 2015); Diyarbakır (Pala et al., 2018), and Kahramanmaraş (Tursun and Kantarcı, 2006) provinces, in the mean of diversity. However, the density estimates of the species reported from the later regions are incompatible. This result indicates the differences among the ecological conditions, size of the irrigated land, farming practices, and performance of the harvesting machines of the mentioned regions.

CONCLUSIONS

This study shows the weed seeds contaminants of the wheat produced in Bingöl province in 2022. 1 kg of wheat grown in this region is found to be comprised 58.5 g (5.8%) of weed seeds. Based on the prevalence frequencies of the observed seeds, *R. arvensis L, H. spontaneum, G. tricornutum, C. arvensis, S. arvensis* and *Vicia sp*., relatively, are the species with highest impact. Our results, display the need for more efficient weed controlling strategies in which biological characteristics of the weeds, and ecological conditions of the growing area are fully accounted.

REFERENCES

Ateş, E., 2022. Determination of the critical period of weed control in wheat (*Triticum aestivum* L.) and investigation of the control methods of wild mustard (*Sinapis arvensis* L.). Malatya Turgut Özal University, Graduate Education Institute, Unpublished Doctoral Thesis, 130 p., Malatya.

Ateş, E., Üremiş, İ. (2018). Determination of germination temperatures of *Sinapis arvensis* L. and *Avena sterilis* L. International Journal of Agricultural and Natural Sciences 1(2): 154-159

Ateş, E., Üremiş, İ. (2020). Determination of weed species and their frequency and density in wheat cultivation areas of Şanlıurfa province, Turkey. Harran Journal of Agricultural and Food Science, 24 (1), 33-43.

Buhler, D.D. (2003). Weed biology, cropping systems, and weed management. Journal of Crop Production, 8(1-2), 245-270.

Dönmez, H.B., Hatipoğlu, R. (2020). Effects of poisonous plants and and preventive measures for for the animal poıisoning in grasslands. Euroasia Journal of Mathematics, Engineering, Natural & Medical Sciences, *7*(9), 49-55.

Esim, R. T., Çoruh, İ. (2021). Determination of the weed species, density and frequency in wheat fields in Bingöl province. Atatürk University Journal of Agricultural Faculty, 52(3), 335-343.

Food and Agriculture Organization (FAO). (2024). Online Edition: “Food and agriculture data”. https://www.fao.org/faostat/en/#home

Gökalp, Ö., Üremiş, İ. (2015). Determination of weed seeds in wheat grain in Mardin province of Turkey. Journal of Agricultural Faculty of Mustafa Kemal University, *20*(1), 23-30.

HRAC GLOBAL, 2024. Synthetic auxin resistance in wild mustard. http://Hracglobal.Com/Files/Wild-Mustard-Fact-Sheet-June-2024.Pdf, (accessed date: 21.05.2024).

Karlsson, L. M., Milberg, P. (2007). A comparative study of germination ecology of four Papaver taxa. Annals of Botany, 99(5), 935-946.

Oerke, E.C. (2006). Crop losses to pests. The Journal of Agricultural Science, 144(1), 31-43.

Pala, F., Mennan, H., Çığ, F., Dilmen, H. (2018). Determination of weed seeds mixed with wheat product in Diyarbakır. Turk J Agric Res 2018, 5(3): 183-190

Tursun, N., Kantarcı, Z., Seyithanoğlu, M. (2006). Determination of weed seeds contaminating wheat grain in Kahramanmaraş province. KSU. Journal of Science and Engineering 9(2), 2006

Zimdahl, R.L. (2018). Fundamentals of weed science (5th ed.). Academic Press.

ASSESMENT OF SURFACE WATER QUALITY INTENDED FOR IRRIGATION PURPOSES IN THE LOWER VALLEY OF THE KEBIR-EAST WADI (NORTH-EASTERN ALGERIA)

BENRABAH Samia1, HALIMI Fahima2, SEDRATI Nassima3

1,2 Department of Geology, Geology Research Laboratory (GRL), Faculty of Earth Sciences, Badji Mokhtar – Annaba University, P.O. Box 12, 23000, Annaba, Algeria.
3 Department of Geology, Water Resources and Sustainable Development Laboratory, Faculty of Earth Sciences, Badji Mokhtar - Annaba University, P.O. Box 12, 23000, Annaba, Algeria.

hydroannaba@hotmail.fr

ABSTRACT

The study area located in the lower valley of the Kebir East watershed is included in a rich environment in natural resources forming part of the El Kala National Park (P.N.E.K) with various RAMSAR classification. A number of water quality indices are being used for assessing irrigation water including: sodium adsorption ratio (SAR), sodium percentage (Na %), potential salinity (PS), Kelly ratio (KR), permeability index (PI), potential salinity (PS), permeability index (PI), Magnesium hazard (MH), Synthetic Harmful Coefficient (K) and Irrigation Coefficient (Ka). All of the strategies used in this investigation yielded similar and complementary results. The lower valley of the Kebir East wadi waters are highly mineralized, as evidenced by total dissolved solids and electrical conductivity. Based on the Doneen categorization, the permeability index indicates that higher values correspond to higher quantities of HCO3 and Na+. Because of high potential salt level (over 50%) and magnesium hazards (above 5 meq/l), the waters in the study region are considered unsuitable for agricultural use.

Key words: Kebir east watershed, wetland, irrigation purposes, mineralization, SAR, salinization.

INTRODUCTION

Because to weathering of rocks and organic matter from chemical fertilizers, the Kebir East (Mafragh) watershed's use for agriculture has resulted in a decline in water quality (Zaidi FK, et al.2015, Bhat NA, et al.,2016). Optimizing the safe use of this wadi water for agricultural uses is the objective of this research and improving the effectiveness of how land resources are used. This research is based on a number of classical. This model's parameters are EC, Na+, Cl−, SAR, and HCO_3 (Meireles et al. 2010). This research can contribute to improving the ecosystem's condition by making a balance between the need for environmental flows and the use of water for consumptive applications (drinking water, industry, and irrigated agriculture) (Matthess, G.1982, EPA. 2005).

MATERIALS AND METHODS

Study area

According to the watershed agency (2000) the Kebir East wadi includes many tributaries, along with Bougous, Guerguour, Boulatane, and Bounamoussa (Hedjal S., Zouini D., Benamara A. 2018). With 750 mm of interannual rainfall, the wadi contributes around 28 Hm^3 over a period of a year. The terrain is mountainous, with 1,041 meters as the highest point, and is covered with oak, cork, and maquis woods (El Tarf forests protection agency, 2019) (Figure.1).

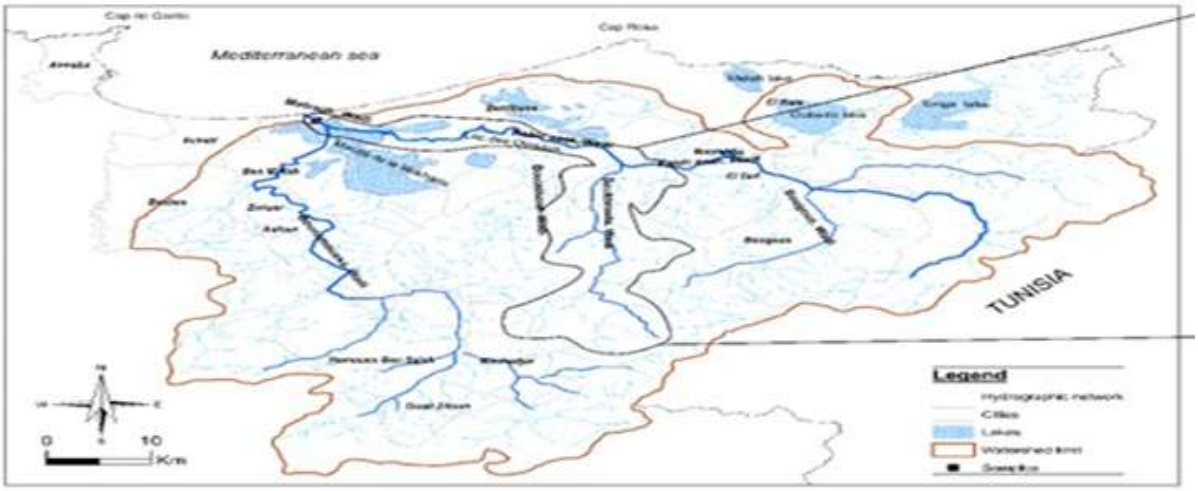


Figure. 1. Map of the study area location

The database that is currently accessible in the Boutheldja meteorological station (longitude Est 8° 12' 22" latitude Nord 36° 47' altitude 20m, code:03-17-01). spans 20 years, from 2000 to 2020. The subhumid Mediterranean climate of the study region is marked by an average annual precipitation of 756 mm. This climate is characterized by an effective infiltration rate of around 13% and evapotranspiration of roughly 63%, the predicted yearly runoff is 186 mm (24%). December had the lowest recorded temperature (-0.6°C), July and August have the highest temperature (46°C). December and January have the highest monthly air humidity levels. An estimate of the yearly average wind speed is 27 m/s.

The geological investigation was completed by Marre. A and Vila J.M (Vila J.M., 1980. Marre A., 1992). These formations belong to the Tell-Algerian region of northeastern Algeria, which extends from the Constantinian area to the Algerian-Tunisian border. The alternation of sandstone, clay-gravelly, and sandstone colluvium forms older geologic formations (Marre A., 1992). The tectonic position is indicated by four faults that are subparallel to the Kebir East wadi.

Used data

The surface water samples from the 2023 campaign are listed on the table below (Table.1). They were taken upstream along the Kebir east wadi (El Mafragh) at eight (08) different points, spaced a few hundred meters apart. pH, EC, DO, NH4, PO4, NO2, NO3, TA, TH, TDS, Ca2+, Mg2+, K+, Na+, Cl-, and SO4 are the parameters that were studied in this research. To guarantee a sufficiently good quality, the charge balance error (CBE) was computed and a 5% margin of error was considered appropriate for CBE values. Mg, Ca, Na, and K rank highest among the cations, whereas HCO3, SO4, Cl, and NO3 rank lowest among the anions. Ca-Mg-HC03 facies or water type is detected. Statistical description (range, mean and standard error) of physicochemical parameters was determined and shown in (Table.1).

Table.1 Statistics on the parameters analyzed in the Kebir East wadi water (El Magragh).

	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	HCO_3^-	SO_4	Cl^-	NO_3	PO_4	NO_2	NH_4	EC	TDS	Turb
Max	180	212	180	8.3	672,23	376,8	286.41	65.09	0,52	1,33	2.1	1972	1183.2	29
Min	100	129.65	54	0,55	167.95	139.55	156.41	18	0,01	0,06	0,45	559	335.4	18,57
average	149.31	163.85	78.16	2.38	444.09	236.82	194,57	35.03	0.11	0.61	1.17	965	604.65	22,47
SD	26.54	29.51	39.03	2.51	185.85	76,81	15,68	8,05	0,17	0,43	0.61	414.8	247.77	3,51

The results show that all parameters (except Mg, SO_4, NH_4, NO_3 and NO_2) do not exceed the standards allowed for surface water, including the maximum values of Ca^{2+}, Na^{+}, K^{+}, HCO_3, TDS, Cl^-, NO_3, and CE. The concentration ranges for magnesium and sulphate ions are respectively 212 mg/l to 129.65 mg/l and 376,8 to 139.55 mg/l, carbonate dissolution is suggested as the cause of the high magnesium content. The potential sources of SO_4 ions in the study area may be derived from agricultural activities (fertilizer inputs) and/or domestic sewage. Furthermore, the application of chemical fertilizers to agricultural land in the research region is the source of organic matter ions, represented by NO3, NH4, PO4 and NO2, which range from 65.09 to 18 mg/l, from 2.1 to 0.45 mg/l, from 0.52 to 0.01 mg/l and from 1.33 to 0.06 mg/l respectively (Sani, A., et al, 2020). Kebir East wadi water is classified as slightly troubled water (5>NTU<30) based on its turbidity, which indicates the presence of suspended particles in the water and fluctuates from 26 to 18.57 NTU.

Irrigation water assessment methods

Agriculture is the main economic activity in this region. Most of the water required for this purpose comes from surface water, notably from the Kebir East wadi (El Mafragh). In this case, various parameters, including the kind of salt and the overall concentration in the water, were created to assess the water's suitability for irrigation (Wilcox, L.V. 1955). The sodium percent (Na%) is a commonly employed measure to assess water suitability for irrigation (Hendry MJ, Wassenaar LI. 2000). Water containing more than 60% salt can lead to sodium buildup, which will deteriorate the physical characteristics of the soil (Adams S.S., et al 2001, Datta PS, Tyagi SK.1996). Additionally, an overabundance of sodium mixed with carbonate results in the production of alkali soils. The Na% is calculated with the formula below:

$$\boldsymbol{Na}\,\% = \frac{\boldsymbol{Na}}{\mathbf{Mg + Ca + K + Na}}\ \mathbf{100}\%$$

The salinity of water is often measured in terms of electrical conductivity (EC) or total dissolved solids (TDS). The quantity of dissolved ions in water is determined by the salinity hazard (Singh, A.K.; et al. 2008). Because harmful substances dissolved in water can damage plants, total dissolved solids (TDS) should be taken into consideration, particularly when it comes to irrigation water (Olivier Montreuil. 2008). Intense agricultural practices that result in the buildup of salts are the primary source of the high TDS value in the water.

A further essential variable in assessing whether irrigation water is desirable is the sodium adsorption ratio (SAR). Irrigation water with a high SAR value has a detrimental impact on crop productivity and soil fertility. As a result, Na ions replace the Ca and Mg ions in the soil, which is unfavorable for agricultural purposes (Richards, L.A. 1954). The SAR is proposed by Richards (1954) and defined as:

$$\boldsymbol{SAR} = \frac{\mathbf{Na}}{\sqrt{(\mathbf{Ca + Mg})/\mathbf{2}}}$$

The parameter-based Permeability Index (PI) measures the permeability of soil, or the ability of water to pass through it. In terms of soil porosity, the concentration of Na+, Ca2+, Mg2+, and HCO3 in water defines the soil profile (Doneen., 1962). Additionally, prolonged usage of rich water on Ca2+, Mg2+, Na+, and HCO3 decreases soil aeration. Irrigation water was categorized in 1962 by Doneen using the permeability index (PI), which took into account the soil's Na^{+}, Ca^{2+}, Mg^{2+}, and HCO_3 levels. It was defined as:

$$\boldsymbol{PI} = \frac{\boldsymbol{Na} + \sqrt{\mathbf{HCO3}}}{\mathbf{Ca + Mg + Na}}\ \mathbf{100}\%$$

According to the PI, water may be divided into three classes: class I (100% maximum permeability), which is appropriate for irrigation; class II (75% maximum permeability), which is somewhat appropriate; and class III (25% maximum permeability), which is not appropriate for irrigation.

The amount of soil structure damage caused on by magnesium in irrigation water is indicated by the magnesium hazard (MH) or magnesium adsorption ratio (MAR) (Bauder, T.A.,2011).

Alkalinity of the soil is caused by a high concentration of Mg2+ ions in groundwater. The infiltration capacity of the soil is also decreased due to a significant quantity of water adsorbed between the magnesium and clay particles, which is bad for crops. Whereas groundwater with a value of MH < 50 is appropriate for irrigation, groundwater with a value of MH > 50 is hazardous. The following formula is used to determine the MH:

$$\boldsymbol{MH} = \frac{\mathbf{Mg}}{\boldsymbol{Ca} + \boldsymbol{Mg}}\ \mathbf{100}\%$$

An essential measure for assessing irrigation water quality based on Na^{+} concentration in comparison to Ca^{2+} and Mg^{2+} concentrations was introduced by KELLY (1963) named the Kelly ratio (KR). Water is not appropriate for irrigation if Kelly's index (KR > 1) is more than 1, indicating an overabundance of sodium in the water (Kelley, W.P., 1940, 1963). Consequently, water that has a KR of less than one (KI <1) is more appropriate for irrigation. The value of this measure is expressed in terms of the ratio of Na^{+} concentrations (meq/L), the sum of Ca^{2+} and the Mg^{2+} (meq/L). The following formula is used to compute this ratio :

$$\boldsymbol{KR} = \frac{\mathbf{Na}}{\mathbf{Ca} + \mathbf{Mg}}$$

The concentration of bicarbonates and carbonate ions in water is the objective of residual sodium bicarbonate (RSBC). Water with a high RSBC therefore has a high pH, which makes the soil it irrigates unusable and causes sodium carbonate to build on the soil. To calculate the RSBC the formula below is used:

$$\boldsymbol{RSBC} = \boldsymbol{HCO}\mathbf{3} - \boldsymbol{Ca}$$

The concept of residual alkalinity is typically employed to forecast how water's chemical composition will change over time. Bicarbonate, carbonate, calcium, and magnesium ions are involved in residual sodium carbonate (RSC) or residual alkalinity (RA), which is described using the formula below:

$$\boldsymbol{RSC} = (\boldsymbol{HCO}\mathbf{3} + \boldsymbol{CO}\mathbf{3}) - (\boldsymbol{Ca} + \boldsymbol{Mg})$$

In the case that the RSC is positive, precipitation occurs in an alkaline manner, conversely, if the RSC is less than zero, precipitation occur in a neutral saline way (Szabolcs, I.; Darab, 1964).
– Following the addition of sulphates associated with the gypsum precipitation, the residual alkalinity becomes positive; this is the neutral saline manner with sulfate dominances.
– Even with the addition of sulphates, the residual alkalinity becomes negative; in this case, the process is known as the neutral saline process with a chloride dominance.

Another metric that's frequently used to describe the quality of water for irrigation is potential salinity, which is represented by ions such as sulfates and chlorides (Marlet, S., et J.O. Job, 2006). There are three established classes: PS <3 indicates exceptional quality, 3 to 5 denotes fair quality, and 5 and higher denotes unsuitable for use. The formula below is used to compute the PS:

$$\mathbf{PS} = \boldsymbol{Cl} + \frac{\mathbf{1}}{\mathbf{2}}\boldsymbol{SO4}$$

We also used the Irrigation Coefficient (Ka) and Synthetic Harmful Coefficient (K) approaches to assess the water quality of the Boukhroufa wadi for irrigation. These coefficients represent the water's alkali and salt hazards. TDS and SAR are added to produce K, which is defined as follows (M is the total dissolved solids in g/l).

$$\boldsymbol{K} = \mathbf{12,4}\boldsymbol{M} + \boldsymbol{SAR}$$

The irrigation coefficient Ka, which was first created in Soviet Russia, is a common method for determining whether water is suitable for irrigation. It is produced through investigating the relative damage of sodium salt and the utmost damage of alkaline solutions. Since the conditions for sodium ion concentrations in our study are lower than those for chlorides, the following formula is employed to get the irrigation coefficient (Ka):

$$\boldsymbol{Ka} = \frac{\mathbf{288}}{\mathbf{5Cl}}$$

RESEARCH RESULTS AND INTERPRETATION

Results of classical indexing methods

The terms "salts, salinity, **electrical conductivity**, or total dissolved solids" are favored in most publications when referring to salinity risk (Marlet, S., et J.O. Job, 2006) One of the most important indicators of irrigation water quality is electrical conductivity, which shows how much of all ion concentrations are dissolved in the water. Water having an EC value above 2250 µs/cm is considered "Fair" or inappropriate and has the potential to significantly impact crop yield or productivity. ‘‘Excellent’’ refers to waters with EC values less than 250 µs/cm (EPA, 2005).

The measured Electrical Conductivity values of the Kebir East (El Mafragh) wadi water range from 1972 to 559 µS/cm for an average of 1007.93 µS/cm. They are classified in two classes, 83% of samples are ranged in class 3 making them doubtful for irrigation purposes, the remaining 17 % of samples fill in class 2 which categorized as good for irrigation use.

The average of **total dissolved solids (TDS)** in the water of Kebir East (El Mafragh) wadi is 604.64 mg/l, with a range of 1183.2 to 335.4 mg/l. Since 66.67% of samples fall into class 2 and have TDS levels that do not exceed 1000 mg/l, irrigation is deemed permissible. Of these, 25% fall into class 1, which is desirable for irrigation usage. Class 3, where water is deemed acceptable, covers the remaining 8.33%.

An overview of water suitability for irrigation is provided by Wilcox's 1955 categorization of water based on the **Sodium percentage (Na%)** (Wilcox LV.1948, 1955). In the water under examination the Na% varies from 29, 36% to 10% for an average of 15.29 %. The Kebir East wadi water samples are classified as excellent for irrigation when 83.33% of them fall into class C1. and as good quality when the remaining 16.66% fall into class C2, this means that depending on the sodium percentage (Na%), the water in the research region is appropriate for irrigation (Figure.2).

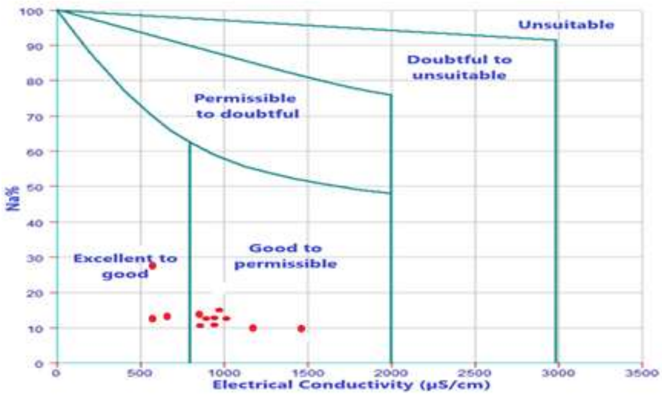


Figure.2 Classification of irrigation water quality based on EC and % Na (Wilcox 1955)

The infiltration and permeability hazard (IPH) or permeability index (PI) defined by Donen in 1962 is calculated for Boukhroufa water samples (Doneen., 1962). The range of PI values is 35.81 to 18.03 meq/l. Doneen's classification results in 58.33% of water samples being classified as class III (PI<25%), meaning that the water is not appropriate for irrigation. This is because the content of Na, Ca, Mg, and HCO3 in irrigation water has a significant impact on soil permeability (Figure.3).

The remaining 41.66% belong to Class II (75%), which makes irrigation with them slightly suitable. Elevated PI values are associated with elevated Na^{+} and HCO_3 levels, may be caused by cation exchange and carbonate dissolution.

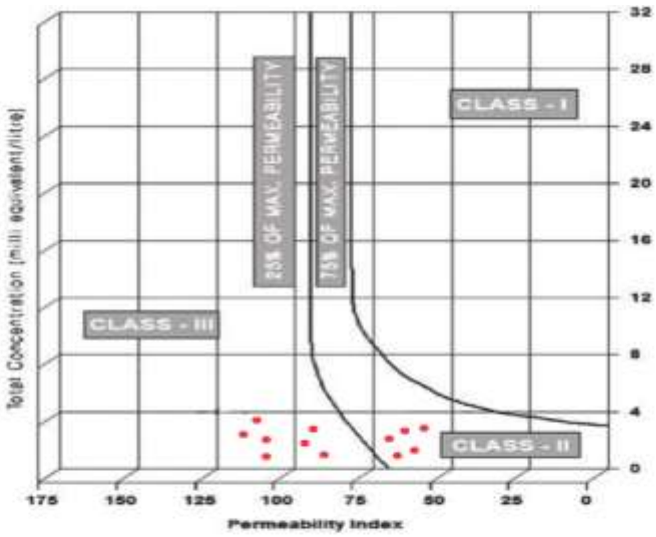


Figure.3 Classification of irrigation water quality based on Doneen diagram (Doneen 1962)

Based on the **salt adsorption ratio (SAR),** water samples taken from the research region are excellent for irrigation. The graphical representation of the sodium absorption ratio (SAR) and the electrical conductivity makes it possible to deduce that all the samples belong to the class (C3) of medium to poor quality in terms of the EC, and in class (S1) of excellent quality in terms of the SAR (Richards, L.A. 1954). Consequently, on well-drained soils, irrigation with Kebir East (El Mafragh) wadi water is appropriate for crops that can tolerate salts, although salinity has to be managed. (Figure.4).

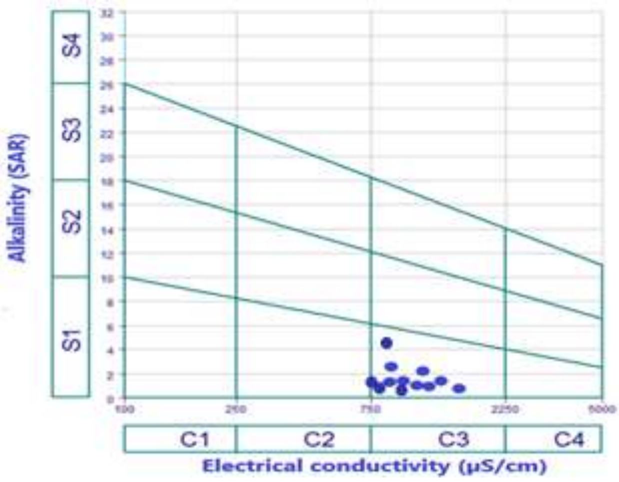


Figure.4 Classification of irrigation water quality based on EC and SAR (Richards 1954)

Also, the classification of water for irrigation purposes can be determined graphically by plotting SAR and EC values on **US Salinity Laboratory diagram (USSL).** The representation (Figure.5) demonstrates that 75% of the samples are grouped in C3S1and 25% in C2S2. This indicates that all of the samples were categorized as low salinity categories based on SAR. In addition, based on EC 25% of samples in medium salinity categories and 75 % in high salinity categories. With these findings, soils with adequate drainage may be irrigated using water from the study area. The USSL diagram indicates that the risk of salinization was not expected in the tested irrigation water samples, and all of the samples could be considered as good medium category of irrigation water.

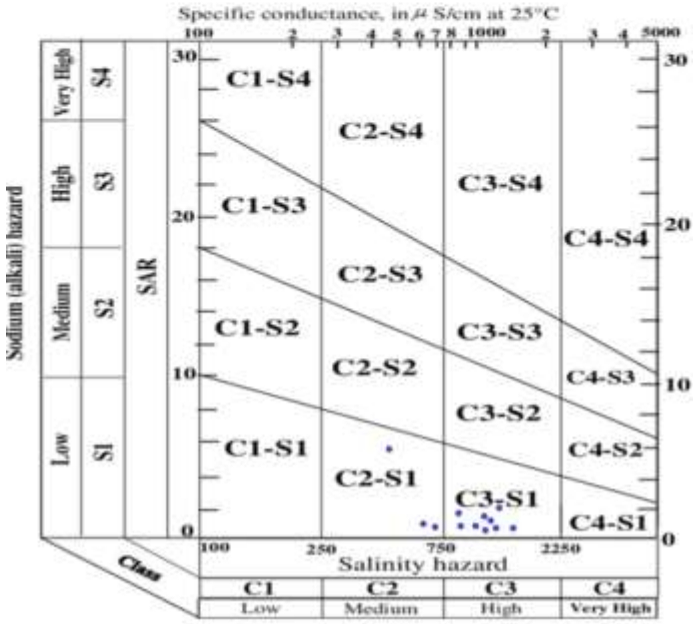


Figure.5 Representation of Kebir East wadi water samples on USSL diagram

The water samples' **Residual Sodium Bicarbonate (RSBC)** values vary from 6.03 mg/L to -6.06 mg/L. A limited amount of CO3 was present in the samples, however the range of HCO3 ions is 672.23 mg/l to 167.95 mg/l. Since more than 50% of the samples have positive RSBC values, the amount of carbonate and bicarbonate in the sample is greater than the amount of dissolved magnesium and calcium ions. Of the water samples, 91.67% are deemed suitable for irrigation, while 8.33 are considered marginal for use in agriculture. Due to their high bicarbonate content and to precipitate insoluble magnesium and calcium, they increase the SAR value and the percentage of sodium (Szabolcs, I.; Darab, C. 1964).

Residual alkalinity, or residual sodium carbonate (RSC), was taken into consideration as an alternate method in the evaluation of irrigation water quality in order to assess the sodization and alkalinization risk of these waters (Szabolcs, I.; Darab, C. 1964). The neutral saline process is operating in the Boukhroufa wadi water, as shown by the negative estimated RSC findings with chloride predominating. This water falls into class 1(RSC<1.25) where water is considered good for irrigation use with a low risk of soil degradation.

With an average of 64.42%, the **magnesium hazard (MH)** of the samples varied from 77.75 to 55.14 percent. In 58.33% of Boukhroufa wadi water samples in the research region had an MH more than 50 making it doubtful for irrigation, the remaining 41.66 % of samples are classed in C3 (>65) where water is unsuitable for irrigation.

The average **Kelly's Ratio (KR)** in water samples was found to be between 0.42 and 0.11 for an average of 0.19. Since all KR values were determined to be below the allowed limit of 1.0, the water in the research region is categorized in C1 class, meaning that it is appropriate for irrigation. Kelley, W.P., 1963).

Table.5 Used approaches for irrigation water

Parameters	Units	Samples (%)	Water quality	Effects on soil and crops
CE	µs/cm	83	*Doubtful*	*Mineralisation excess, the rate of water uptake by plants decreases*
		17	*Good*	
TDS	mg/l	66.67	Permissible	
		25	Desirable	
		8.33	Acceptable	
SAR	/	100	Excellent	Nothing dangerous or damaging to report
Na%	%	83.33	Excellent	
		16.66	Good	
PI	%	58.33	*Unsuitable*	*high levels of Na and HCO3, affect soil porosity,*
		41.66	*Slightly suitable*	
MH/MAR	%	58.33	*Doubtful*	*Soil structure damage, reduice infiltration capacity, adverse effects on crops*
		41.66	*Unsuitable*	
KR	/	100	Suitable	Nothing dangerous or damaging to report
RSBC	meq/l	91.67	Satisfying	*Causes infertile soils, deposition of sodium carbonate*
		8.33	*Marginal*	
RSC	meq/l	100	Good	Nothing dangerous or damaging to report
PS	meq/l	100	*Injurious to unsatisfactory*	*Hight level of chlorides and sulfates ions*
K	/	100	Excellent	Nothing dangerous or damaging to report
Ka	/	100	Excellent	*Relative damage of sodium salt on soil and crops*

Three classes are used to categorize the water quality for irrigation based on **potential salinity (PS)**, PS below three, water is good to excellent, below five water is good to harmful and beyond five water is harmful to unsatisfactory. With a mean of 7.49 meq/l the PS values in the

Kebir East wadi water samples ranged from 10.44 to 5.88 meq/l. 100% of the samples fall into class 3, where they are considered injurious to unsatisfactory for irrigation due to their potential salinity (more than 5).

For Kebir East water samples the synthetic harmful coefficient (K) has a mean of 9.51 and a range of 15.7 to 6.31. Since all of the samples are greater than 25 (Class 1) they are all deemed excellent for irrigation use.
Four classifications constitute the **irrigation coefficient (Ka)** categorization method for water. Water quality with a Ka value of more than 18 is excellent for irrigation, whereas water quality with a Ka value of less than 1.2 is inappropriate for irrigation purposes. The Ka values for Kebir East water vary from 13.03 to 7.12 meaning that all water samples (100%) are listed in class 1, which are excellent for use in agriculture.
The table below includes a list of every method used in this investigation (Table.5) where the findings are consistent and complementary.

CONCLUSION

The appropriateness of 08 irrigation water samples in the research region was evaluated based on hydrochemical results and categorization using a variety of methods. These approaches have been used to examine the Kebir East (El Mafragh) wadi's water quality for irrigation purposes. This wadi's waters are often highly mineralized. According to the data, 83% of samples are doubtful for irrigation due to increased EC values. Based on the permeability index, more than half of the water samples are not appropriate for irrigation, where soil porosity is affected by elevated Na^+ and HCO_3 values. Additionally, because of the increased risk of magnesium hazards (MH) 58.33% and 41.66% of total samples are doubtful and unsuitable respectively for agricultural use. As a result, higher concentrations of sulfates and chlorides can have a negative impact on crops and alter the structure of soils (reducing infiltration capacity). The water used for irrigation facilitates the deposition of sodium carbonate and results in soil infertility, as demonstrated by the analysis of the water quality index of residual sodium bicarbonate (RSBC), which shows that 91.66% and 8.33% of the total samples of our study area fall under satisfying and marginal category respectively. For the potential salinity (PS), 100% of samples are injurious to unsatisfactory for irrigation purposes.
The average Kelly's Ratio (KR) indicates that all samples tested were determined to be below the permissible limit of 1.0, indicating that the water in the research region has an equilibrium of sodium, calcium, and magnesium ions. The sodium adsorption ratio (SAR) and sodium % data show that there is no sodium risk in the study region water and no hazard of alkalization when using this few sodium content in the water for irrigation.
Based on the wadi's sample locations, upstream is considered to have higher levels of mineralisation, permeability index (PI), sodium percent (Na%) and potential salinity (PS) than downstream. This indicates that the primary process governing the chemistry of the groundwater in this region is the weathering of minerals. The contact between water and rock, which specifically results in cation exchange, is most likely the cause of the unusually high concentration of Na+ ions in wadi water. Furthermore, upstream agricultural practices including the use of agrochemicals might raise the salt concentration. Therefore, from the

perspective of irrigation, water samples obtained downstream are preferred to those collected upstream due to the dilution phenomena that occurred, as demonstrated by the findings of all the used parameters. In terms of viewpoint, both field monitoring and experimental research to follow and anticipate the geochemical and physical development of soils irrigated by various types of water in the region are required.

REFERENCES

1. Nora Allouani, Samia Benrabah, Hamza Bouguerra, Fahima Halimi, Salah Eddine Tachi; Origin of spring water chemistry and its appropriateness for drinking requirements in a semi-arid environment (Ain Larbi, Algeria). Water Supply 1 July 2024; 24 (7): 2506–2517. doi: https://doi.org/10.2166/ws.2024.148

2. Matthess, G. (1982) The Properties of Groundwater. Department of Environmental Science, John Wiley and Sons Inc., New York, 406 p.

3. Environmental Protection Agency, Office of Water. 2005. Handbook for Developing Watershed Plans to Restore and Protect our Waters. Washington, DC United States Environmental Protection Agency, 400 p.

4. AYERS R.S., WESTCOT D.W. 1985. Water quality for agriculture. FAO Irrigation and Drainage paper. No. 29. Rev 1. Rome. FAO. ISBN 92-5-102263-1 pp. 174.

5. AYERS, R. S.; WESTCOT, D. W. A Qualidade da Água na Agricultura. 2. ed. Campina Grande: UFPB, 1999. 218 p. (Estudos FAO: Irrigação e Drenagem, 29)

6. Vila J.M., 1980. La chaîne alpine d'Algérie orientale et des confins algéro-tunisiens, Thèse de Doctorat Es-sciences Naturelles. Université Pierre et Marie - Curie, Parie VI, pp 282-296.

7. Marre A., 1992. Le Tell oriental Algérien de Collo à la frontière Tunisienne, Etude géomorphologique. Vol. 1 & 2, Office des Publications Universitaires. Alger, Algérie.

8. Zaidi FK, Nazzal Y, Jafri MK, Naeem M, Ahmed I (2015) Reverse ion exchange as a major process controlling the groundwater chemistry in an arid environment: a case study from northwestern Saudi Arabia. Environ Monit Assess 187:607. https://doi.org/10.1007/s10661-015-4828-4

9. Islam, M., Azad, A., Ara, M., Rahman, M., Hassan, N., Swarnokar, S. and Rabeya, I. (2016) Environmental Study on a Coastal River of Bangladesh with Reference to Irrigation Water Quality Assessment: A Case Study on Shailmari River,

Khulna. *Journal of Geoscience and Environment Protection*, **4**, 41-64. doi: 10.4236/gep.2016.410003.

10. Bauder, T.A., Waskom, R.M., Sutherland, P. and Davis, J.D. (2011). Irrigation Water Quality Criteria Crop Series|Irrigation module. Fact Sheet No. 0.506. Colorado State University, Extension, USA

11. Marlet, S., et J.O. Job, 2006. Processus et gestion de la salinité des sols. In : Tiercelin, J.R. Traité d'irrigation, seconde édition. Tec & Doc Lavoisier. ISBN-13: 978-2743009106.

12. Subramani, T., Elango, L., & Damodarasamy, S. R. (2005a). Groundwater quality and its suitability for drinking and agricultural use in Chithar River basin, Tamil Nadu, India. Journal of Environmental Geology, 47, 1099–1110. doi:10.1007/s00254-005-1243-0.

13. Li, P., Zhang, Y., Yang, N. *et al.* Major Ion Chemistry and Quality Assessment of Groundwater in and Around a Mountainous Tourist Town of China. *Expo Health* **8**, 239–252 (2016). https://doi.org/10.1007/s12403-016-0198-6

14. Bhat NA, Bhat AA, Nath S, Singh BP, Guha DB (2016) Assessment of Drinking and Irrigation Water Quality of Surface Water Resources of South-West Kashmir, India. J Civil Environ Eng 6: 222. doi:10.4172/2165-784X.1000222

15. Adams S.S., Titus R., Pietersen K., Tredoux G., Harris C., 2001.Hydrochemical characteristics of aquifers near Sutherland in Western Karoo, South Africa. Journal of Hydrology, v. 241, p. 91-103. Geochem. 19, 19–27.

16. Hendry MJ, Wassenaar LI. 2000. Controls on the distribution of majors ions in pure waters of a thick surficial aquitard. Water Resources Research, 36: 503-513

17. Datta PS, Tyagi SK (1996) Major ion chemistry of groundwater in delhi area: chemical weathering processes and groundwater fowregime. J GeolSoc India 47:179–188

18. Singh, A.K.; Mondal, G.C.; Kumar, S.; Singh, T.B.; Tewary, B.K.; Sinha, A. Major ion chemistry, weathering processes and water quality assessment in upper catchment of Damodar River basin, India. Environ Geol. 2008, 54, 745–758.

19. Li, P.; Wu, J.; Qian, H. Assessment of groundwater quality for irrigation purposes and identification of hydrogeochemical evolution mechanisms in Pengyang County, China. Environ. Earth Sci. 2013, 69, 2211–2225.

20. Meireles, A.; Andrade, E.M.; Chaves, L.; Frischkorn, H.; Crisostomo, L.A. A new proposal of the classification of irrigation water. Rev. Cienc. Agron. 2010, 413, 349–357.

21. Richards, L.A. Diagnosis and Improvement of Saline Alkali Soils. In Agriculture Handbook; US Department of Agriculture: Washington, DC, USA, 1954; Volume 60, p. 160.

22. Wilcox LV (1948) The quality of water for irrigation use. In: Egfrdg (Eds.), US Department of Agriculture, Technical Bulletin. Washington DC, UK, p.19

23. Wilcox LV (1955) Classification and use of irrigation waters. U.S. US Department of Agriculture Circular 969, Washington, U.S. Department of Agriculture, DC, p. 19

24. Doneen, L.D. Notes on Water Quality in Agriculture. Published as a Water Science and Engineering; Department of Water Sciences and Engineering, University of California: Oakland, CA, USA, 1964; Volume 4001.

25. Kelley, W.P. (1963). Use of saline irrigation water. Soil Sci., 95, 385–391. https://doi.org/10.1097/00010694-196306000-00003

26. Szabolcs, I.; Darab, C. The Influence of Irrigation Water of High Sodium Carbonate Content of Soils. In Proceedings of the 8th International Congress of ISSS, Bucharest, Romania, 31 August–9 September 1964; Volume II, pp. 803–812.

27. Adimalla, N.; Taloor, A.K. Hydrogeochemical investigation of groundwater quality in the hard rock terrain of South India using Geographic Information System (GIS) and groundwater quality index (GWQI) techniques. Groundw. Sustain. Dev. 2020, 10, 100288.

28. Hedjal S., Zouini D., Benamara A. 2018. Hydrochemical assessment of water quality for irrigation: A case study of the wetland complex of Guerbes-Sanhadja, North-East of Algeria. Journal of Water and Land Devel opment. No. 38 p. 43–52. DOI: 10.2478/jwld-2018-0041.

29. Olivier Montreuil. Relation entre l’ordre des bassins versants, l’organisation spatiale et le fonction nement hydrologique et hydrochimique des zones humides riveraines. Hydrologie. Agrocampus- Ecole nationale supérieure d’agronomie de rennes, 2008. Français.

30. Simsek, C. and Gunduz, O. (2007) IWQ Index: A GIS-Integrated Technique to Assess Irrigation Water Quality. Environmental Monitoring and Assessment, 128, 277-300. http://dx.doi.org/10.1007/s10661-006-9312-8

31. Sani, A., Musa, M. U., Adamu, U.K., Sanda, A.R, Adam, I.A and Almu, H. River water quality assessment and suitability for irrigation in northern sudan savanna, ecological zone of nigeria. International Journal of Environment and Pollution Research. 2020. Vol.8, No.3, pp.30-40,
32. Abbasnia, A.; Radfard, M.; Mahvi, A.H.; Nabizadeh, R.; Yousefi, M.; Soleimani, H.; Alimohammadi, M. Groundwater quality assessment for irrigation purposes based on irrigation water quality index and its zoning with GIS in the villages of Chabahar, Sistan and Baluchistan, Iran. Data Brief 2018, 19, 623–631.

33. Singh, K.K.; Tewari, G.; Kumar, S. Evaluation of groundwater quality for suitability of irrigation purposes: A Case Study in the UdhamSingh Nagar, Uttarakhand. J. Chem. 2020, 2020, 6924026.

34. Ouarani M, Bahir M, Mulla DJ, Ouazar D, Chehbouni A, Dhiba D, Ouhamdouch S, El Mountassir O. Groundwater Quality Characterization in an Overallocated Semi-Arid Coastal Area Using an Integrated Approach : Case of the Essaouira Basin, Morocco. *Water*. 2020 ; 12(11):3202. https://doi.org/10.3390/w12113202

USE OF HAZARDOUS ELEMENT RISK ASSESSMENT INDICES TO EVALUATE THE WATER QUALITY OF SAZLIDERE DAM LAKE (İSTANBUL, TÜRKIYE)

Cem Tokatlı

Trakya University, Evrenos Gazi Campus, İpsala Vocational School, Department of Laboratory Technology, Edirne / Türkiye
**Corresponding Author: cemtokatli@trakya.edu.tr*

ABSTRACT

Certainly, environmental pollution and its impact on freshwater habitats are critical issues that require attention. Large dams are constructed worldwide for various purposes, including flood control, water supply, hydroelectric power generation and recreation and reservoirs may alter the aquatic ecology and river hydrology both upstream and downstream, affecting water quality and quantity. The Sazlıdere Dam Lake is located in the north part of Marmara Region and meets the drinking water needs of İstanbul Province of Türkiye. Therefore, it has an important effect on the health of many people. In this research, concentrations of 9 PTEs including chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb), manganese (Mn) and boron (B) were investigated in water of Sazlıdere Dam Lake. Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI) were used to evaluate the water qualities of investigated locations in terms of PTEs contamination. According to the applied heavy metal risk assessment indices, water of Sazlıdere Dam Lake was recorded as "Low heavy metal contamination (HPI < 100)" in terms of applied HPI and "Low contamination (HEI < 10)" in terms of HEI.

Keywords: Sazlıdere Dam Lake, İstanbul Province, Risk Assessment Indices

INTRODUCTION

Sazlıdere Dam Lake is located in the İstanbul Province of Türkiye and it meets the drinking water needs of the region. Therefore, the reservoir has a very important effect on the health of many people living in the İstanbul Province (Anonymous, 2020). The reservoir, which has a capacity of approximately 92 million m³, was built on the Sazlıdere Stream in 1996 by State Hydraulic Works (DSİ). The body volume of the Sazlıdere Dam Lake, which is a rock body fill type, is 1.880.000 m³ and its height from the riverbed is 48 meters. The reservoir volume is about 92 hm³ and the reservoir area is about 11.81 km² at the normal water level (Anonymous, 2020; http://suyonetimiormansu.gov.tr).

Heavy metal risk assessment indices are useful tools to evaluate the water quality in terms of human health and Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI) are among the most commonly used risk assessment indices (Tokatlı et al., 2021; Varol and Tokatlı, 2022; Haque et al., 2022; Jannat et al., 2022; Mutlu et al., 2023; Mia et al., 2023;

Tokatlı et al., 2023; 2024; Yüksel et al., 2024). The aim of this research was to evaluate the water quality of Sazlıdere Dam Lake in terms of potentially toxic elements (PTEs) by using Heavy Metal Pollution Index (HPI) and Heavy Metal Evaluation Index (HEI).

MATERIAL AND METHOD

Study Area and Collection of Samples

In the present study, levels of PTEs in water of Sazlıdere Dam Lake were determined and the data were evaluated by using HPI and HEI. Surface water samples were collected from 3 stations (input of dam 1 – SD1, input of dam 2 – SD 2 and output of dam – SD3) selected on the Sazlıdere Dam Lake in rainy (winter) season of 2022 (Figure 1).

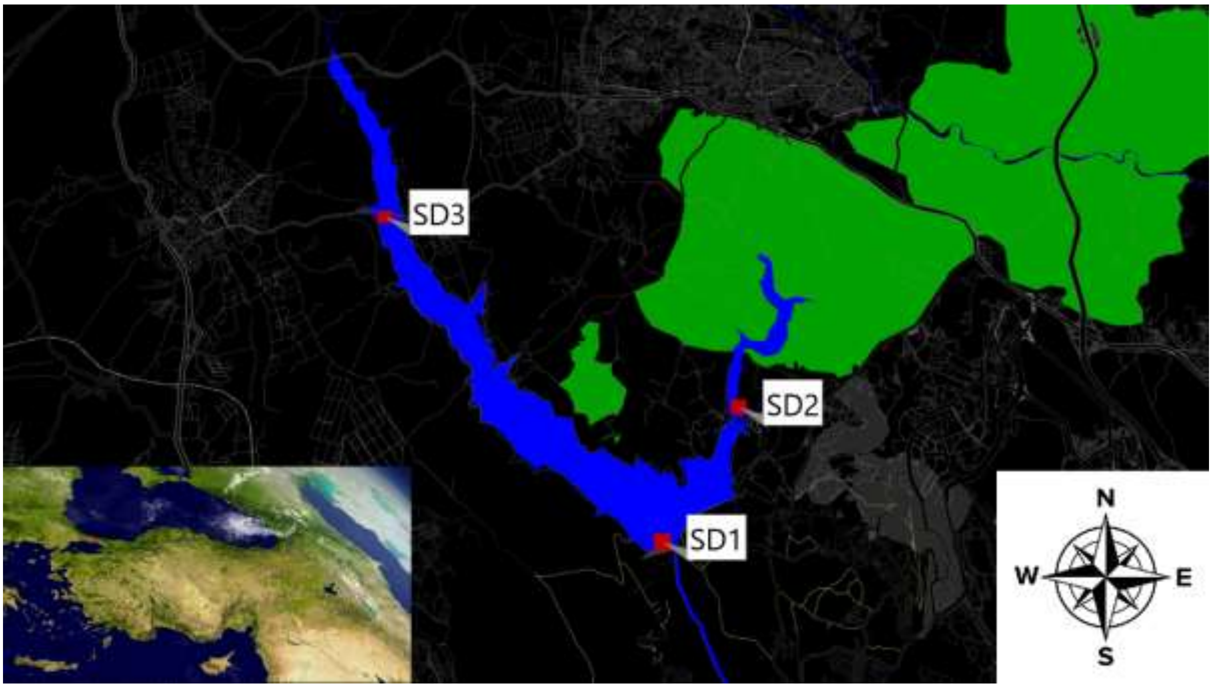


Figure 1. Study area and selected stations

Chemical Analysis

For determination of chromium (Cr), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), lead (Pb), manganese (Mn) and boron (B) concentrations in water of Sazlıdere Dam Lake, samples of one liter were adjusted to pH 2 by adding 2 ml of HNO_3 into each. Afterwards, all the samples were filtered (cellulose nitrate, 0.45 µm) in such a way as to make their volumes to 50 ml with ultra-pure water. The element levels in water samples were determined by using the "Agilent 7700 xx" branded Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) device in Trakya University Technology Research and Development Application and Research Center (TÜTAGEM). The center has an international accreditation certificate within the scope of TS EN / ISO IEC 17025 issued by TÜRKAK (representative of the World Accreditation Authority in Turkey). The element analyses were recorded as means triplicate measurements (APHA, 1992; EPA, 2001).

Calculation of Risk Assessment Indices

Heavy Metal Pollution Index (HPI) (formulas 1 and 2) (Mohan et al., 1996) and Heavy Metal Evaluation Index (HEI) (formula 3) (Edet and Offiong, 2002) are being calculated according to the following formulas:

$$HPI = \frac{\sum_{i=1}^{n} W_i Q_i}{\sum_{i=1}^{n} W_i} \quad (1)$$

$$Q_i = \sum_{i=1}^{n} \frac{M_i}{S_i} \times 100 \quad (2)$$

$$HEI = \sum_{i=1}^{n} \frac{H_C}{H_{MAC}} \quad (3)$$

“Qi” is the sub – index of the toxic element, “Wi” is the unit weight of the ith parameter, “Mi” is the monitored values of toxic metals, “Si” is the standard values of the parameter and n is the number of parameters considered (WHO, 2011). Water quality ratings for applied HPI are given in Table 1.

"Hc" is value observed for each parameter and "Hmac" indicates the value of maximum admissible concentration (MAC) for each parameter (WHO, 2011). Water quality ratings for applied HEI are given in Table 1.

Table 1. Water quality ratings for indices

Value	Rating of Water Quality	Usage Possibilities
Heavy metal pollution index (HPI)		
< 100	Low heavy metal contamination	Suitable
> 100	High heavy metal contamination	Not suitable
Heavy Metal Evaluation Index (HEI)		
< 10	Low contamination	Suitable
10 – 20	Medium contamination	Not suitable
> 20	High contamination	Not suitable

RESULTS AND DISCUSSION

As a result of applied toxicological risk assessment indices, water of Sazlıdere Dam Lake was found as "Low heavy metal contamination (HPI < 100)" in terms of applied HPI and "Low contamination (HEI < 10)" in terms of HEI and it was recorded as "Suitable for consumption and use" (Figure 2). The monomial risk ranking of elements were found as in terms of HPI:

As > Cd > Cr > Ni > Pb > Mn > B > Cu > Zn; while the risks in terms of HPI were as follows: Cr > B > Ni > As > Cd > Mn > Pb > Cu > Zn (Figure 3). It was also determined that the spatial pollution degrees of the investigated locations on the Sazlıdere Dam Lake were as follows: SD1 > SD2 > SD3, in general.

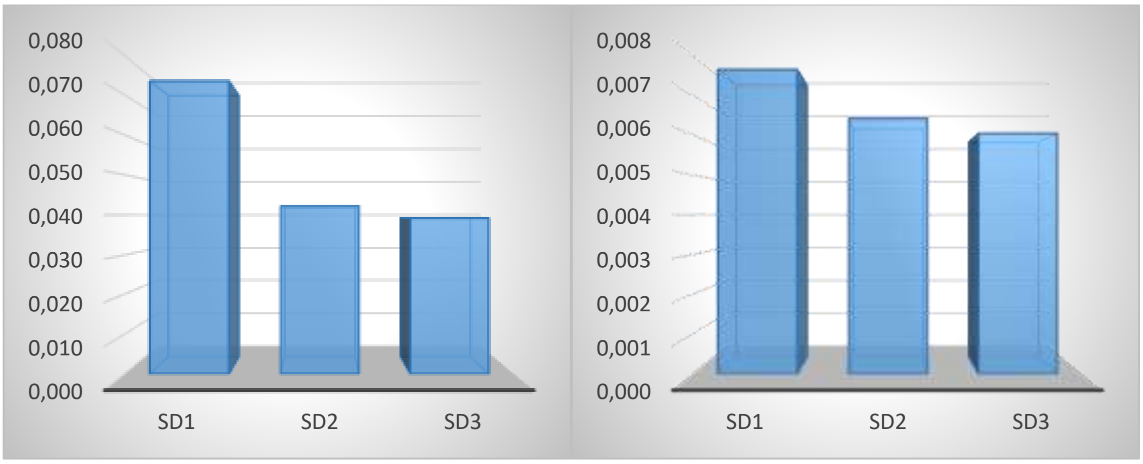


Figure 2. Results of applied HPI (left) and HEI (right)

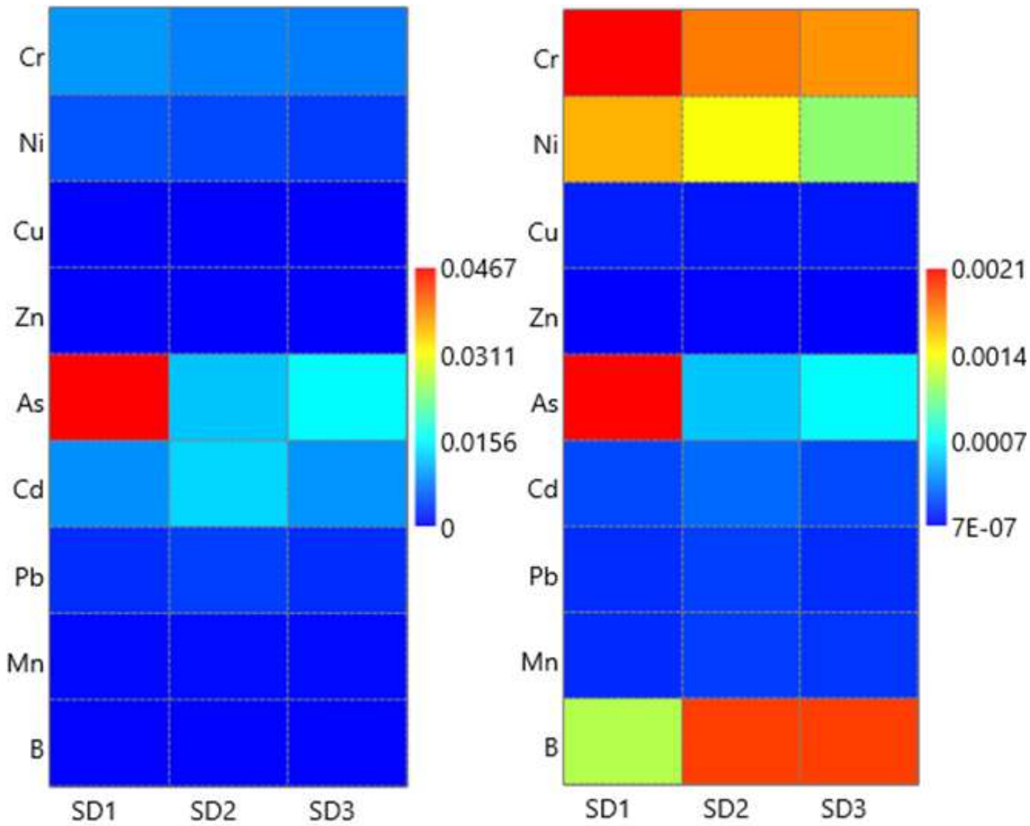


Figure 3. Monomial results of applied HPI (left) and HEI (right)

CONCLUSIONS

In this research, water quality of Sazlıdere Dam Lake located in the İstanbul Province of Türkiye was evaluated in terms of PTEs contamination by using HPI and HEI. According to the results of applied toxic metal risk assessment indices, Sazlıdere Dam Lake was found as "Low heavy metal contamination" in terms of applied HPI and "Low contamination" in terms of HEI. The data of the present research also reflects the importance, applicability and necessity of the use of different toxic metal risk assessment indices together on evaluation of surface water ecosystems.

ACKNOWLEDGEMENTS

This research was supported by Trakya University Scientific Research Projects (project no: 2022/63).

REFERENCES

Anonymous, 2020. 2020 Environmental status report of İstanbul Province. Republic of Türkiye, İstanbul Governorship, Provincial Directorate of Environment and Urban Management.

APHA (American Public Health Association). 1992. Standard methods for the examination of water and wastewater. In A.E. Greenberg, A.E., Clesceri, L.S. and Eato, A.D. (eds.) American Public Health Association, 18th ed., Washington, U.S.A.

Edet, A.E., Offiong, O.E., 2002. Evaluation of Water Quality Pollution Indices for Heavy Metal Contamination Monitoring. A Study Case from Akpabuyo-Odukpani Area, Lower Cross River Basin (Southeastern Nigeria). GeoJournal, 57, 295–304.

Environmental Protection Agency (EPA) METHOD 200.7. 2001. Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry.

Haque, J., Siddique, A. B., İslam, S., Ali, M. M., Tokatlı, C., İslam, A., Pal, S. C., İdris, A. M., Malafaia, G., İslam, A. R. T., 2023. Effects of covid-19 era on a subtropical river basin in bangladesh: heavy metal(loid)s distribution, sources and probable human health risks. Science of the Total Environment, https://doi.org/10.1016/j.scitotenv.2022.159383.

http://suyonetimiormansu.gov.tr

Jannat, J. N., İslam, K. M. S., İslam, H. M. T., İslam, M. S., Khan, R., Bakar, S. M. A., Varol, M., Tokatlı, C., Chandra, P. S., İslam, A., İdris, A. M., Malafaia, G., Islam, A. R. T., 2022. Hydrochemical assessment of fluoride and nitrate in groundwater from east and west coasts of Bangladesh and India. Journal of Cleaner Production, https://doi.org/10.1016/j.jclepro.2022.133675.

Mia, Y., İslam, A. R. T., Jannat, J. N., Jion, M. M. F., Sarker, A., Tokatlı, C., Siddique, A. B., İbrahim, S. M., Senapathi, V., 2023. Identifying factors affecting irrigation metrics in the Haor Basin using integrated Shannon's Entropy, fuzzy logic and automatic linear model. Environmental Research, 226: 115688.

Mohan, S.V., Nithila, P., Reddy, S.J. 1996. Estimation of Heavy Metal in Drinking Water and Development of Heavy Metal Pollution Index. Journal of Environmental Science and Health, Part A, 31 (2), 283–289.

Mutlu, E., Tokatlı, C., Islam, A. R. T., İslam, S., Muhammad, S., 2023. Water quality assessment of Şehriban Stream (Kastamonu, Türkiye) from a multi-statistical perspective. International Journal of Environmental Analytical Chemistry, https://doi.org/10.1080/03067319.2023.2197114.

Tokatlı, C., Mutlu, E., Arslan, N., 2021. Assessment of the potentially toxic element contamination in water of Şehriban Stream (Black Sea Region, Turkey) by using statistical and ecological indicators. Water Environmnet Research, 93: 2060–2071.

Tokatlı, C., Onur, Ş. G., Dindar, M. B., Malafaia, G., Islam, A. R. T., Muhammad, S., 2023. Spatial-temporal variability and probabilistic health risk assessment of fluoride from lentic ecosystem, Türkiye. International Journal of Environmental Analytical Chemistry, https://doi.org/10.1080/03067319.2023.2198645.

Tokatlı, C., Mutlu, E., Ustaoğlu, F., Islam, A. R. T., Muhammad, S., 2024. Spatiotemporal Variations, Health Risk Assessment, and Source Changes of Potentially Toxic Elements in Potamic Water of the Anday Stream Basin (Türkiye), Black Sea Region. Environmental Monitoring and Assessment, 196: 420.

Varol, M., Tokatlı, C., 2022. Seasonal variations of toxic metal(loid)s in groundwater collected from an intensive agricultural area in northwestern turkey and associated health risk assessment. Environmental Research, 204, Part A: 111922.

World Health Organization (WHO), 2011. Guidelines for Drinking-water Quality. World Health Organization Library Cataloguing-in-Publication Data, NLM classification: WA 675.

Yüksel, B., Ustaoglu, F., Aydın, H., Tokatlı, C., Topaldemir, H., Islam, S., Muhammad, S., 2024. Appraisal of Metallic Accumulation in the Surface Sediment of a Fish Breeding Dam in Türkiye: A Stochastical Approach to Ecotoxicological Risk Assessment. Marine Pollution Bulletin, 203: 116488.

THE ONGOING FOURTH INDUSTRIAL REVOLUTION AND THE CIRCULAR ECONOMY

Nicoleta SEICARU BUCATA, Liviu MARCUTA

Faculty Of Management And Rural Development, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
nicoleta.seicaru@doctorat.usamv.ro

marcuta.liviu@managusamv.ro

ABSTRACT

We are already facing the consequences of using the linear economy model. Many alarm signals have been raised that this model involves the excessive exploitation of the environment with the aim of increasing consumption and implicitly profit. This cannot go on indefinitely. However, we see a hope given by the Circular Economy model supported by the fourth industrial revolution. This refers to new technologies from artificial intelligence, digitization, renewable energies, sustainability. Many researchers see a singularity point soon, a point where the changes brought to society by developments in technology will occur at an unimaginable pace. This paper argues (based on some concrete examples) that the solution to sustainable development is the Circular Economy based on the innovations of the fourth industrial revolution. In the era of IND 4.0 the Circular Economy will be digital, automated and augmented or it will not exist at all.

Key Words: History of Economics, IND 4.0, Circular Economy, singularity, Succes Stories for Circular Economy

INTRODUCTION

One must understand the past before making assumptions about the future. The history of our species had not always a linear development, but rather a winding evolution, thriving civilizations, followed by devastating wars, weakened ecosystems, then new developments of the world and above all a steep dynamic. It is also a story of technological advances with close relationship between societal structures and access to material and energy resources.
From time to time, the discovery of an innovative tool changes everything and leads to a new stage of civilization. Some consider the first technological revolution to be the Upper Paleolithic Revolution, about 50,000 years ago. The invention of tools, the ability to control and use fire led to the emergence of behavioral modernity. These events formed the basis for the first waves of migration, when people left Africa and began to settle on other continents.
There are many examples of similar pulses in history: the agricultural techniques developed the first civilizations to rise from the river plains of the Near East and Asia 13,000 years ago; the invention of modern weapons; the print technology (Guttenberg); the mechanical inventions of the Renaissance period (Leonardo da Vinci); the commercial revolution of the 16th century when Europeans developed through colonialism and the scientific revolution of the 17th century with a systematic distribution of knowledge. These all represent radical transformations that have altered the course of civilization in part due to technological booms. However, we must note that these transformations have not always been 100% positive.

Important innovations drive technological revolutions, and this leads to a series of subsequent changes. Different discoveries influence each other and cause the transition from a type of society to another. These changes can sometimes have dramatic effects. In just a few decades, production systems, education systems, political regimes and dominant business models can be radically transformed.
The First Industrial Revolution (end of the 18th century - beginning of the 19th century) was characterized by the transition from manual to mechanized production. Inventions such as the steam engine, textile mills, and ironworking technology led to increased productivity and the development of industry.
The Second Industrial Revolution (late 19th century - early 20th century) brought major technological innovations such as electricity, the telegraph, the telephone, the internal combustion engine, and mass production. This Revolution led to a significant increase in industrial production and the expansion of transportation networks.
The Third Industrial Revolution (20th century - early 21st century), also known as the Digital Revolution, was marked by the development of information and communication technologies such as personal computers, the Internet, mobile technology and Industrial automation. It has had a significant impact on the way people work, communicate and live.
The Fourth Industrial Revolution (present - near future) is based on the convergence of digital, physical and biological technologies such as artificial intelligence, advanced robots, 3D printing, biotechnology and renewable energies. This has the potential to fundamentally change the way society works, including in areas such as health, transport, manufacturing and the environment.

The Ongoing Fourth Industrial Revolution (IND 4.0)

The first three Industrial Revolutions were characterized by major innovations in areas such as manufacturing, energy, communications, and automation. The Fourth Industrial Revolution focuses on the convergence of digital, physical, and biological technologies. The fourth Industrial Revolution (IND 4.0) is based on technologies such as artificial intelligence, the Internet of Things (IoT), big data analysis (big data), blockchain, advanced robotics, virtual and augmented reality, 3D printing, biotechnology, renewable energies, etc. These technologies combine to transform economic and social sectors.
A central feature of the Fourth Industrial Revolution is digitization, which involves the transformation of processes and activities into digital forms, and connectivity, which enables communication between devices, people and systems. This can lead to efficiency optimization, personalized choice, better resource management, etc.
Robots and intelligent systems are becoming increasingly capable of performing complex tasks and making decisions based on data and context. This can lead to increased productivity in the industry, but it can also have significant implications for the workforce human work.
The fourth Industrial Revolution can bring significant transformations in the labor market. Certain jobs will disappear or transform, while new opportunities will emerge in technology, design, data management, and more. Thus, adapting and re-educating the workforce becomes essential.
With the increasing adoption of advanced technologies, industries such as healthcare, manufacturing, transportation, agriculture, renewable energies and many others are likely to undergo significant transformations. IND 4.0 can also contribute to solving major challenges such as climate change and global health.
It is important to note that discussions of the Fourth Industrial Revolution are developing, and its ultimate impact on society and the economy will continue to evolve as technologies advance and are widely adopted.

Linear Economy vs. Circular Economy

Linear economy is a system in which people buy a product, use it, and then throw it away. The term linear refers to the straight progression that a product can follow, with a beginning, a middle and an end. There is no thought along the line regarding recycling or reuse. This model is characterized by a high volume of new manufacturing. The linear economy is a polluting system that can hurt nature and the climate. It causes loss of biodiversity.

Linear economic thinking has been around for most of the 20th century. It is based on the desire to make products and offer services for the lowest price. Raw materials are extracted from nature at the lowest cost, turned into products with the least amount of labor, and sold at the highest price.

This linear “take-make-use-dispose” economy is not working. We are extracting virgin raw materials at a pace so fast that they cannot be replenished. We are already having problems extracting some critical raw materials that are in constant demand. At the same time, the amount of waste keeps growing. Global waste generation is expected to increase by 70% by 2050.

What is a Circular Economy?

The ideal type of economy is a circular one that tries to minimize waste and use resources again and again. A Circular Economy is based on three principles: reduce, reuse, and recycle. In a Circular Economy, waste is minimized, natural resources are conserved, and pollution is reduced. The first step is to eliminate the over-extraction of raw materials by using secondary raw materials salvaged from existing products.

Secondary raw materials are products that have already been used in the manufacturing process. These are products designed so that at the end of their lives, they can be upgraded, repaired or go through a new manufacturing process. They also are more durable and resilient.

How the fourth industrial revolution will help Circular Economy

Although there are many discussions and policy initiatives regarding the Circular Economy, it is usually ignored that IND4.0 provides the technological, economic and social framework in which the Circular Economy will flourish or fail. For example, it is estimated that the implementation of the Circular Economy in the European Union will create up to 1.2-3 million new jobs by 2030. However, the report seems to ignore the fact that the activities necessary in preparing for reuse, debugging and disassembly would have to be automated and robotic or otherwise could not be economically viable. In the era of IND4.0 the Circular Economy will be digital, automated and augmented or it will not exist at all.

On the other hand, discussions of IND4.0 usually focus on the resource and labor productivity advantages, the radical changes in business models and the social challenges involved. It is rarely discussed (except when avoiding the linear "business as usual" approach) that IND4.0 will simultaneously stimulate and accelerate resource depletion and pollution in an era where Earth is rapidly approaching or has even exceeded its characteristic limits. IND4.0 will coexist with the Circular Economy or accelerate environmental degradation and the potential collapse of ecosystems and human societies.

The question that arises is whether IND4.0 and the Circular Economy will converge, thus providing not only more efficient resource management but also a sustainable future for all? Or will IND4.0 evolve along the linear "business as usual" model, leaving the Circular Economy a passing fad, leading to faster depletion of resources, accelerating environmental degradation and deepening inequalities? A realistic response must involve the transformation of the waste management sector.

Artificial Intelligence for Waste Reuse

Artificial intelligence (AI) is revolutionizing the way we manage waste, transforming it from a problem into a valuable resource. Some examples of how AI is being used for waste reuse are: Waste Sorting and Recycling (AI-powered Sorting, Fill Level Monitoring), Waste Management Optimization (Route optimization, Predictive maintenance, Capacity planning), Waste-to-Energy, Circular Economy (Material recovery, Product design optimization), Waste tracking, Waste prevention.
Some examples for Waste Reuse AI-driven are:

- ZenRobotics from Finland which uses AI-powered robotic arms to sort and separate waste materials. These robots are equipped with advanced sensors and AI algorithms to identify and pick out valuable materials like metals, wood, and plastics from mixed waste streams.
- AMP Robotics from United States which employs AI and computer vision to automate the sorting of recyclables. Their systems can identify and separate various types of materials, including plastics, metals, and paper, from a conveyor belt in recycling facilities.
- Tomra Sorting Solutions from Norway uses AI-enhanced optical sorting systems for waste management. These systems utilize machine learning algorithms to improve the identification and separation of different types of waste, such as plastics, glass, and metals.
- Bin-e from Poland. The bins are equipped with sensors and machine learning algorithms to identify and segregate waste accurately.
- Waste2Wear from Netherlands that uses AI to facilitate the transformation of plastic waste into sustainable textiles. Their AI-driven platform matches plastic waste sources with manufacturers that can recycle the waste into fabric.
- Lasso Loop from United States that uses AI in its home recycling appliance that cleans, sorts, and processes recyclable materials. The AI system identifies the type of waste and ensures it is properly cleaned and prepared for recycling. By streamlining the recycling process at the source (homes), Lasso Loop increases recycling rates and improves the quality of materials sent to recycling facilities.
- Everledger from United Kingdom that employs AI and blockchain technology to track and verify the provenance of recycled materials, such as plastics and metals. This ensures the transparency and authenticity of recycled products.

By leveraging AI, we can significantly improve waste management practices, conserve resources, and create a more sustainable future.

CONCLUSION

The Circular Economy has a key role in the Fourth Industrial Revolution (IND4.0), with numerous correlations. How they interact could decide the future of society. With all the discussions about the Circular Economy and the fourth Industrial revolution, it is rarely recognized that they are deeply interconnected, and in real life they cannot be separated.

Circular Economy is not just a fad, but a necessity. Our planet faces several environmental challenges, and the Circular Economy offers a viable solution for a more sustainable future. Moving to a Circular Economy will require a change in mindset at all levels of society, but the benefits are clear and worth the effort.

REFERENCES

Antonis Mavropoulos, Anders Waage Nilsen (2020). Industry 4.0 and Circular Economy: Towards a Wasteless Future or a Wasteful Planet?, 9781119699279, John Wiley & Sons Ltd.

What is the linear economy? https://www.eib.org/en/stories/linear-economy-recycling

Barles, S. (2014). History of waste management and the social and cultural representations of waste. In: The Basic World Environmental History, vol. 4 (eds. M. Agnoletti and S.N. Serneri), 199–226. Cham: Springer

Hollins, O., Lee, P., Sims, E. et al. (2017). Towards a Circular Economy – Waste Management in the EU; Study IP/G/STOA/FWC/2013-001/LOT 3/C3. EPRS | European Parliamentary Research Service.

Ellen MacArthur Foundation. The Circular Economy in detail. https://www.ellenmacarthurfoundation.org/explore/the-circular-economy-in-detail

ACCENTURE (2016). The Circular Economy could unlock $4.5 trillion of economic growth, finds new book by Accenture. ACCENTURE Newsroom. https://newsroom.accenture.com/news/the-circular-economy-could-unlock-4-5-trillion-of-economic-growth-finds-new-book-by-accenture.htm

Oliver Cann (2016). $100 trillion by 2025: the digital dividend for society and business. World Economic Forum, 2016. https://www.weforum.org/press/2016/01/100-trillion-by-2025-the-digital-dividend-for-society-and-business

Oxford Economics (2017). Digital Spillover - Measuring the True Impact of the Digital Economy. Huawei.

Tearfund (2019). No Time to Waste Tackling the Plastic Pollution Crisis before It's Too Late. Tearfund.

Enerdata (2018). Global energy statistical book 2019. https://yearbook.enerdata.net

REN21 (2019). Renewables 2019 Global Status Report. Paris: REN21

World Bank. Energy use (kg of oil equivalent per capita)|Data. https://data.worldbank.org/indicator/EG.USE.PCAP.KG.OE

IEA (2019). World Energy Outlook 2019 Executive Summary; WORLD ENERGY OUTLOOK, 11. International Energy Agency.

Ritchie, H. and Roser, M. (2017). Meat and Dairy Production. Our World in Data

de Vries, M. and de Boer, I.J.M. (2010). Comparing environmental impacts for livestock products: a review of life cycle assessments. Livestock Science 128 (1–3): 1–11

Tuomisto, H.L. and Teixeira de Mattos, M.J. (2011). Environmental impacts of cultured meat production. Environmental Science and Technology 45 (14): 6117–6123.

https://aquademica.ro/wp-content/uploads/2020/11/RO-Anca-Turtoi-Holland-Circular-Hotspot.pdf

HEAVY METAL CONTENTS AND POLLUTION STATUS OF SOILS UNDER DIFFERENT LAND USE TYPES IN SULTAN MARSHES

Selma YAŞAR KORKANÇ[1], Mustafa KORKANÇ[2], Ahmad Farid AMIRI[3]

[1] Niğde Ömer Halisdemir University, Engineering Faculty, Department of Environmental Engineering, Niğde, Türkiye
[2] Niğde Ömer Halisdemir University, Engineering Faculty, Department of Geological Engineering, Niğde, Türkiye

[3] Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences, Niğde, Türkiye

Corresponding author e-mail: sykorkanc@ohu.edu.tr

ABSTRACT

The aim of this study is to determine some heavy metal contents and contamination status of soils under different land use types around the Sultan Marshes ecosystem. For this purpose, 36 topsoil (0-20 cm) samples were taken from some of the different land use types in the wetland ecosystem (rangeland. agriculture, marsh and dry lake) and Cr, Pb, Fe, Zn, Cu, Co, Mn, Cd, Mo, As, Ni) were determined by ICP MS and ICP OES devices. Relationships between heavy metals were found by Pearson correlation analysis. Pollution status of the soils according to land use patterns was evaluated according to the contamination factor (C_f) the degree of contamination (C_d). Soils taken from the Southern Marshes had higher values than other land use types in terms of average Cr, Fe, Zn, Co, Cu and Ni concentrations. According to C_f, dry lake soils were exposed to extreme pollution in terms of As and moderate pollution in terms of Cd. Soils under other land use types have been exposed to low-moderate pollution in terms of various heavy metals according to this factor. According to the C_d factor, rangeland and agricultural areas were exposed to significant levels of pollution in terms of Cd, and the dry lake area was exposed to a very high level of pollution in terms of As. Monitoring and taking measures to control heavy metal pollution in soils under all land use types is necessary for ecosystem sustainability.

Keywords: Sultan marshes, land use types, heavy metal, pollution

INTRODUCTION

Wetlands are valuable areas with important ecosystem services. To meet various human needs, wetland areas can be drained and converted into agricultural land, or used for purposes such as settlement, livestock farming, reed cutting, etc. These and similar changes in land use are among the most significant environmental changes affecting ecosystem functions and biodiversity (Ferreira et al., 2016). Land use changes resulting from intense human activities and urbanization have a significant impact on soil quality (Xia et al., 2011). Human activities that involve changing land use can also alter the elemental composition of soils (Wang and Xu, 2014).

Land use changes occur in wetlands for various purposes. One of the issues that degrade soil quality in wetland ecosystems and pose risks to living organisms is heavy metal contamination. Therefore, it is extremely important and necessary to determine the characteristics of heavy metal distribution in wetland soils, assess pollution risks and sources, and identify measures to protect the health of wetland ecosystems (Wang et al., 2019). In a study by Nuralykyzy et al. (2021) on metal levels in different land uses, they found that concentrations of Cu, Cr, Ni, and As were higher in agricultural areas compared to orchards, pastures, and industrial areas. However, Bai et al. (2010) also noted that soils under cultivation along a typical plateau lake in China showed relatively lower heavy metal concentrations compared to abandoned cultivated wetland soils.

Many wetlands in Turkey have undergone significant changes due to various pollution factors, such as heavy metals (Türker and Vymazal, 2021). For the health of wetland ecosystems, it is necessary to properly establish land use policies. The Sultan Marshes wetland ecosystem is one of the areas in Turkey that has been subjected to the effects of human pressure on wetlands. In a study conducted in the Sultan Marshes, Demirezen and Aksoy (2004) reported heavy metal pollution (Cd, Pb, Ni, Cr, Cu, and Zn) in sediments, water, and some reed plants. Aksoy et al. (2005) revealed that Cr concentrations were particularly high in plants and sediment. Furthermore, Demirezen and Aksoy (2006) discussed biological indicators for Fe and Mn pollution and detected heavy metal contamination in *Phragmites australis*, *Ranunculus sphaerosphermus* plants, and sediment. In another study conducted in the Sultan Marshes, researchers investigated the sources of heavy metal pollution in and around the Sultan Marshes, noting that various heavy metal concentrations were derived from geological, mining, industrial, residential, traffic, and agricultural sources (Yalçın et al., 2007)."

This wetland has been subjected to land use changes since the 1940s, with some sections being drained and converted into agricultural land (DSI 1970; Gürer 2004; Gürer and Yıldız 2008; Yaşar Korkanç et al., 2022). Yaşar Korkanç et al. (2022) also noted that changes in land use in the Sultan Marshes ecosystem have affected the carbon stock and various soil properties. Existing research on heavy metal pollution in different wetland ecosystems has shown that the degree of heavy metal contamination varies among wetland types and that the potential ecological risk of heavy metals in inland wetlands is increasing (Sandilyan and Kathiresan, 2014; Liu et al., 2015). A review of past studies reveals a need for data on whether the differences in land use within the research area have impacted heavy metal concentrations. Identifying changes in selected heavy metals based on land use types, assessing the status of different land use types in terms of heavy metal pollution, determining the ecological risk, identifying measures that can be taken based on the level of contamination, and establishing land management plans will provide a crucial foundation.

The aim of this study is to determine the heavy metal content and pollution status of soils under different land use types in the Sultan Marshes.

MATERIAL AND METHOD

Site Description

The Sultan Marshes wetland is located in the Kayseri-Develi Closed Basin, between the latitudes of 38°12'14"-38°25'49" North and longitudes of 35°09'20"-35°22'20" East. The surface area varies seasonally between 8,000 and 13,000 hectares. The study area is situated on alluvium in the center of the Develi Closed Basin, with an elevation ranging from 1,070 to 1,150 meters above sea level. The average slope is 2% (DSI, 1995; Gürer, 2004). The annual average precipitation is 363 mm, and the annual average temperature is 11°C (Özesmi and Gürer, 2003). The climate type is semi-arid subtropical Mediterranean continental (Aksoy, 2004; Karadeniz, 1995). Generally defined as the Develi Closed Basin, the region features basalt, andesite, and tuffs resulting from the volcanic activities of Mount Erciyes in the north and northwest. In the central part of the basin, as one approaches the wetland, finer-grained alluvium is found, while in the south, thick, massive layers of gray limestone are present. The sedimentary rock units in the Develi Closed Basin extend in an east-west direction, while the magmatic rocks stretch in a narrow strip in a north-south direction (DSI, 1995). The Sultan Marshes National Park and Ramsar Site encompass various habitats, including freshwater and saltwater ecosystems, reed/marsh areas, and surrounding meadow, pasture, and steppe areas (Aksoy, 2004). The land in Sultan Marshes is used for agriculture, grazing, settlement, mining, and other human activities (Yaşar Korkanç et al., 2022).

Methods

The research was designed according to a completely randomized block design. Based on field conditions, the land use types in the wetland ecosystem where soil sampling was conducted were identified as follows: 1. Rangeland areas, 2. Southern Marshlands (Örtülüakar Marshes), 3. Former wetlands that were converted to agricultural land (orchards and farmland) at different times due to DSI projects, and 4. The Dry lake area (around Yay Lake) that became exposed after water receded. Three sampling plots (20x30 m) were selected for each land use type, and disturbed soil samples were taken from the topsoil layer (0-20 cm) at three points within each sampling plot.

In the laboratory, the soil samples, which were air-dried at room temperature, were prepared for analysis by passing them through a 0.2 mm nylon sieve. The soil samples were sent to the laboratories of the General Directorate of Mineral Research and Exploration (MTA), where they were prepared according to the TS ISO14869-1 Standard method (URL 1). The concentrations of Cr, Pb, Fe, Zn, Fe, Mn, Cu, Co, Mo, As, and Ni in the soil samples were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The relationships between heavy metal concentrations in the soils were determined using Pearson correlation analysis with the IBM SPSS 24.0 statistical software package (Zar, 1996). The pollution status of the soils in the study area was assessed using the contamination factor (C_f) ($C_f \leq 1$: low contamination; $1 \leq C_f < 3$: moderate contamination; $3 \leq C_f < 6$: considerable contamination; $C_f \geq 6$: very high contamination) and the degree of contamination (C_d) ($C_d < 11$: low contamination; $11 \leq C_d <$

22: moderate contamination; 22 ≤ C_d < 44: considerable contamination; 44 ≤ C_d < 44: high contamination; C_d ≥ 44: very high contamination) (Hakanson, 1980).

C_f, the contamination factor, was calculated by dividing the heavy metal concentration in the studied soil by the average concentration of that metal in the soil, using the following formula (Hakanson, 1980):

$$C_f = C_{metal}/C_0 \tag{1}$$

In the formula, C_{metal} represents the concentration of the metal in the soil sample, and C_0 is the average background concentration of the metal in soil, according to Turekian and Wedepohl (1961). For the heavy metals in our research, the background reference values according to Turekian and Wedepohl (1961) are Cr=90, Pb=20, Fe=47200, Mn=850, Zn=95, As=13, Cd=0,3 , Co=19, Cu=45, Mo=2,6 ve Ni=68.

Contamination Degree (C_d) were calculated by summing of all pollution factors of a particular basin (Hakanson, 1980). Hakanson's (1980) thresholds for contamination have been modified for your study since it includes 11 heavy metals. The "low contamination" threshold, initially set at 8, has been adjusted to 11, with other threshold ranges revised accordingly.

RESULTS AND DISCUSSION

The Distribution of Heavy Metal Concentrations in Soils According to Different Land Uses

In the Southern Marsland, the concentrations of heavy metals varied as follows: Cr: 91.90-158.60 mg/kg, Pb: 17.10-34.80 mg/kg, Fe: 17,624-32,869 mg/kg, Mn: 443-559 mg/kg, Zn: 58-282 mg/kg, As: 4.5-24.50 mg/kg, Cd: 0.09-1.20 mg/kg, Co: 7.50-13.30 mg/kg, Cu: 21.20-152.20 mg/kg, Mo: 1.90-10.10 mg/kg, Ni: 89.20-204.40 mg/kg. Based on the average values, the heavy metal concentrations in the Southern Marsland were ranked from highest to lowest as follows: Fe > Mn > Ni > Zn > Cr > Cu > Pb > As > Co > Mo > Cd (Figure 1). It was found that in the Southern Marsland, Cr exceeded the background reference values by 39.58%, Pb by 25.72%, Zn by 41.05%, Cd by 80.74%, Mo by 105.56%, and Ni by 101.13%. Cu was at the threshold level, and As was close to the reference value.

In the rangeland soils, the concentrations of heavy metals varied as follows: Cr: 30.80-141 mg/kg, Pb: 17.0-42.90 mg/kg, Fe: 6,631-25,964 mg/kg, Mn: 165-587 mg/kg, Zn: 40-96 mg/kg, As: 6.90-110.40 mg/kg, Cd: 0.09-1.60 mg/kg, Co: 2-11.20 mg/kg, Cu: 13.60-29.60 mg/kg, Mo: 1.30-7 mg/kg, Ni: 23-144.20 mg/kg. The average heavy metal concentrations in the rangeland soils were ranked from highest to lowest as follows: Fe > Mn > Zn > Cr > Ni > As > Pb > Cu > Co > Mo > Cd (Figure 1). It was found that in the pasture soils, the average concentrations of Pb exceeded the background reference values by 23.50%, As by 110.09%, Cd by 158.89%, and Mo by 60.26%.

In the dry lake area soils, the concentrations of heavy metals varied as follows: Cr: 23.80-25.60 mg/kg, Pb: 7.80-17.80 mg/kg, Fe: 6,904-7,374 mg/kg, Mn: 180-198 mg/kg, Zn: 20-32 mg/kg, As: 70.2-120 mg/kg, Cd: 0.30-1.20 mg/kg, Co: 1.50-2.60 mg/kg, Cu: 7.70-10.80 mg/kg, Mo: 0.60-2.10 mg/kg, Ni: 14.20-19.0 mg/kg. The average heavy metal concentrations in the dry lake area soils were ranked from highest to lowest as follows: Fe > Mn > As > Zn > Cr > Ni > Pb > Cu > Co > Mo > Cd (Figure 1). It was found that the average concentrations of Cd in the soils exceeded the background reference values by 114.81%, and As concentrations exceeded by 574.44%

In the agricultural soils, the concentrations of heavy metals varied as follows: Cr: 27.90-169.70 mg/kg, Pb: 8.50-75.20 mg/kg, Fe: 5,626-35,229 mg/kg, Mn: 87-678 mg/kg, Zn: 19-116 mg/kg, As: 0.10-29 mg/kg, Cd: 0.09-1.40 mg/kg, Co: 1.60-15.20 mg/kg, Cu: 10-104.40 mg/kg Mo: 1.60-9.50 mg/kg, Ni: 11.80-198.60 mg/kg. The average heavy metal concentrations in the agricultural soils were ranked from highest to lowest as follows: Fe > Mn > Zn > Cr > Ni > Cu > Pb > As > Co > Mo > Cd (Figure 1). It was found that in the agricultural soils, the average concentrations of Pb exceeded the background reference values by 62.92%, As by 29.55%, Cd by 124.72%, and Mo by 28.21%.

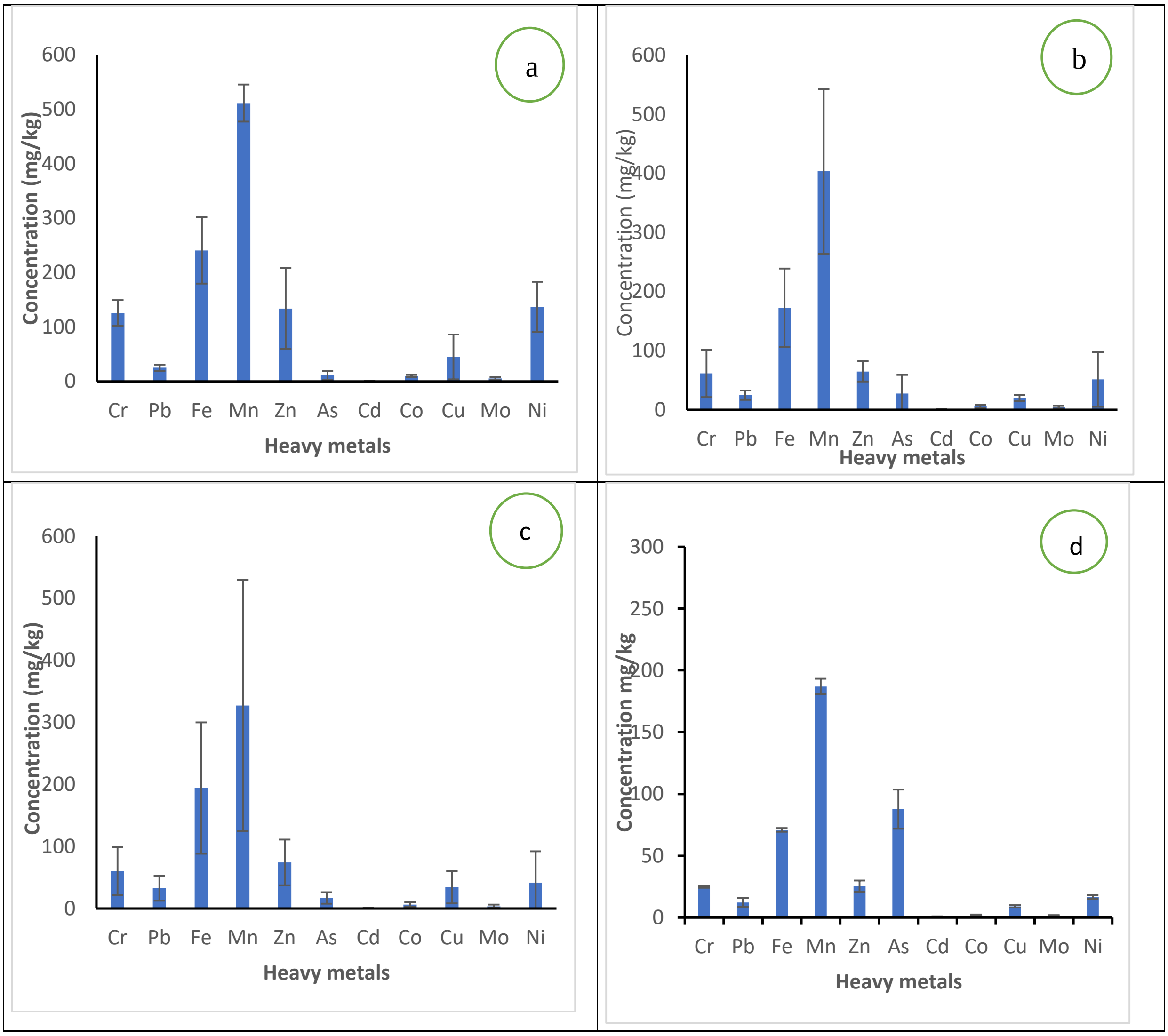


Figure 1. The heavy metal concentrations in soils from different land uses a. **Southern Marshlands** b. Rangelands c. **Farmlands** d. **Dry Lake Area** (Fe concentrations are expressed as percentages in the graph due to their very high levels)

Variation in Correlations Between Heavy Metals According to Land Use Types

In the Southern Marsland, the statistically significant relationships between heavy metal concentrations in the soil were as follows: Cr showed a positive correlation with Fe, Co, and Ni. Fe exhibited a positive correlation with Cr, Mn, Co, and Ni. Mn had a positive correlation with Fe, Co, and Ni. Co was positively correlated with Cr, Fe, Mn, and Ni. Ni showed a positive correlation with Cr, Fe, Mn, and Co (Table 1).

The statistically significant correlations between heavy metal concentrations in the soil of the rangeland area were as follows: Cr showed a positive correlation with Fe, Mn, Co, Cu, Mo, and Ni, while Pb showed a positive correlation only with Zn. Fe exhibited a positive correlation with Cr, Mn, and Co, whereas Mn showed a positive correlation with Cr, Fe, Co, and Ni. Zn showed a positive correlation with Pb, and Co had a positive correlation with Cr, Fe, Mn, Cu, and Ni. Cu showed a positive correlation with Cr, Co, and Ni. Mo exhibited a positive correlation with Cr and Ni, while Ni showed a positive correlation with Cr, Mn, Co, Cu, and Mo (Table 1).

When examining the correlations between heavy metal concentrations in the soils taken from the Dry lake area, it was found that only Zn and As have a significant negative correlation (Table 1).

Table 1. Correlation matrix of soil heavy metals in different land use types

		Cr	Pb	Fe	Mn	Zn	As	Cd	Co	Cu	Mo	Ni
Southern Marshland	Cr	1										
	Pb	-0.162	1									
	Fe	0.924**	-0.007	1								
	Mn	0.553	0.003	0.680*	1							
	Zn	-0.148	0.508	-0.204	-0.429	1						
	As	0.495	0.284	0.582	0.117	-0.051	1					
	Cd	0.459	0.644	0.491	0.469	0.372	0.343	1				
	Co	0.896**	-0.058	0.987**	0.772*	-0.268	0.502	0.491	1			
	Cu	-0.036	0.660	0.054	0.237	0.259	-0.134	0.598	0.060	1		
	Mo	-0.122	-0.126	0.006	-0.011	0.021	0.333	-0.341	0.006	-0.588	1	
	Ni	0.921**	-0.035	0.998**	0.687*	-0.222	0.547	0.480	0.990**	0.050	-0.021	1
Rangeland	Cr	1										
	Pb	-0.115	1									
	Fe	0.679*	0.523	1								
	Mn	0.752*	0.115	0.879**	1							
	Zn	0.264	0.771*	0.661	0.336	1						
	As	-0.390	-0.219	-0.614	-0.538	-0.097	1					
	Cd	-0.240	0.073	-0.290	-0.339	-0.358	-0.090	1				
	Co	0.935**	0.072	0.870**	0.921**	0.376	-0.547	-0.336	1			
	Cu	0.847**	-0.117	0.510	0.587	0.360	0.105	-0.415	0.751*	1		
	Mo	0.702*	-0.435	0.347	0.597	-0.364	-0.592	0.070	0.664	0.326	1	
	Ni	0.986**	-0.244	0.561	0.685*	0.135	-0.306	-0.166	0.877**	0.852**	0.733*	1
Dry lake	Cr	1										
	Pb	-0.549	1									
	Fe	0.544	0.206	1								
	Mn	0.405	0.168	0.632	1							
	Zn	0.408	0.225	0.593	0.122	1						
	As	-0.598	0.264	-0.529	-0.098	-.821**	1					
	Cd	0.595	-0.317	0.389	0.345	0.401	-0.577	1				
	Co	0.405	-0.285	0.439	0.438	0.220	-0.610	0.288	1			
	Cu	-0.113	0.131	0.250	-0.287	0.552	-0.609	-0.041	0.391	1		
	Mo	-0.305	0.204	-0.081	0.611	-0.428	0.360	-0.144	0.328	-0.247	1	
	Ni	-0.383	0.049	-0.664	-0.144	-0.336	0.512	-0.103	-0.626	-0.597	0.223	1
Farmland	Cr	1										
	Pb	0.438	1									
	Fe	0.805**	0.806**	1								
	Mn	0.783**	0.840**	0.910**	1							
	Zn	0.473	0.845**	0.887**	0.753**	1						
	As	0.041	-0.456	-0.276	-0.319	-0.395	1					
	Cd	0.505	0.365	0.577*	0.515	0.540	0.277	1				
	Co	0.961**	0.617*	0.928**	0.892**	0.672*	-0.116	0.537	1			
	Cu	0.273	0.816**	0.560	0.789**	0.588*	-0.399	0.301	0.438	1		
	Mo	0.730**	0.311	0.636*	0.433	0.415	-0.112	0.333	0.677*	-0.074	1	
	Ni	0.961**	0.233	0.619*	0.660*	0.227	0.181	0.419	0.861**	0.172	0.623*	1

*Significant correlations at 0.05 level (2-tailed),

**Significant correlations at 0.01 level (2-tailed)

The statistically significant correlations between heavy metal concentrations in the soils from the agricultural area were as follows: Cr shown a positive correlation with Fe, Mn, Co, Mo, and Ni. Pb shown a positive correlation with Fe, Mn, Zn, Co, and Cu, while Fe exhibited a positive correlation with Cr, Pb, Mn, Zn, Cd, Co, Mo, and Ni. Mn had a positive correlation with Cr, Pb, Fe, Zn, Co, Cu, and Ni, whereas Zn shown a positive correlation with Pb, Fe, Mn, Co, and Cu. Cd shown a positive correlation only with Fe. Co shown a positive correlation with Cr, Pb, Fe, Mn, Zn, Mo, and Ni, while Cu exhibited a positive correlation with Pb, Mn, and Zn. Mo shown a positive correlation with Cr, Fe, Co, and Ni, and Ni exhibited a positive correlation with Cr, Fe, Mn, Co, and Mo (Table 1).

When heavy metal sources are similar or identical, there is typically a significant correlation between their concentrations. The strength of the relationship between heavy metal elements can therefore reflect their source environments (Luo et al., 2018; Zeng et al., 2018). The correlations in this study also suggest that the heavy metals may have originated from a common or similar source. Similarly, Nuralykyzy et al. (2021) found strong correlations between Zn–Cd ($p < 0.01$), Cr–Ni ($p < 0.05$), and Ni–Pb ($p < 0.05$). Additionally, Jiang et al. (2017) reported that Co, Cr, Cu, Pb, and Zn have significant positive correlations with each other, indicating they may have originated from the same sources.

Evaluation of Pollution

Based on the average C_f values, the heavy metals measured in the soil of the Southern Marsland area could be ranked as follows: Mo (2.06) > Ni (2.01) > Cd (1.81) > Zn (1.41) > Cr (1.40) > Pb (1.26) > Cu (1.0) > As (0.86) > Mn (0.60) > Co (0.52) > Fe (0.51). According to the C_f values, 88% of the soil samples from the Southern Marshland area were moderately contaminated with Ni, 100% with Cr, 77% with Mo and Pb, 44% with Zn and Cd, 33% with As, and 11% with Cu. Significant contamination was observed in 22% of samples for Cd and 11% for Cu. Low contamination was found for As, Mn, Co, and Fe.

In the rangelands, the heavy metals ranked by average C_f values were as follows: Cd (2.59) > As (2.10) > Mo (1.60) > Pb (1.24) > Ni (0.75) > Cr = Zn (0.68) > Mn (0.47) > Cu (0.44) > Fe (0.36) > Co (0.30). In this area, 67% of soil samples were moderately contaminated with As, Cr, and Mo; 33% with Cd; 22% with Cr and Ni; 33% of samples were significantly contaminated with Cd; and 11% were highly contaminated with As. Low contamination was observed for Ni, Cr, Zn, Mn, Cu, Fe, and Co.

For the Dry lake area, the heavy metals ranked by average Cf values were as follows: As (6.74) > Cd (2.15) > Pb (0.61) > Mo (0.58) > Cr = Zn (0.27) > Ni (0.24) > Mn (0.22) > Cu (0.20) > Fe (0.15) > Co (0.11). In this area, 78% of soil samples were highly contaminated with As, 22% were significantly contaminated with As, 89% were moderately contaminated with Cd, and 11% were significantly contaminated with Cd. Low contamination was observed for Pb, Mo, Cr, Zn, Ni, Mn, Cu, Fe, and Co.

In the agricultural area, the heavy metals ranked by average C_f values were as follows: Cd (2.25) > Pb (1.63) > As (1.30) > Mo (1.28) > Zn (0.78) > Cu (0.76) > Cr (0.67) > Ni (0.61) > Fe (0.41) > Mn (0.39) > Co (0.31). In this area, 58% of soil samples were moderately contaminated with Pb, 42% with Zn, 67% with As, 50% with Cd, and 33% with Mo. Significant contamination was observed in 25% of samples for Cd, 8% for Pb, and 8% for Mo. Low contamination was observed for Zn, Cu, Cr, Ni, Fe, Mn, and Co.

When the soil samples taken from the Southern Marshland area were evaluated in terms of C_d, it was observed that C_d values range between 4.6 and 18.49, indicating low to moderate contamination. The percentage contribution of each element to the total C_d in the Southern Marshland soils could be ranked from highest to lowest as follows: Mo (15.29%) > Ni (14.96%) > Cd (13.45%) > Zn (10.50%) > Cr (10.38%) > Pb (9.37%) > Cu (7.42%) > As (6.44%) > Mn (4.48%) > Co (3.89%) > Fe (3.80%). Based on C_d values, the soils from Southern Marshland were moderately contaminated with Mo, Ni, Cd, Zn, Cr, and Pb, and were slightly (low) contaminated with other heavy metals.

In the rangeland area, the C_d values of the soil samples ranged from 2.68 to 23.30, indicating low to significant contamination. The percentage contribution of each element to the total C_d in the rangeland soils was as follows: Cd (23.08%) > As (18.72%) > Mo (14.28%) > Pb (11.03%) > Ni (6.71%) > Zn (6.08%) > Cr (6.07%) > Mn (4.23%) > Cu (3.89%) > Fe (3.25%) > Co (2.65%). Based on C_d values, the soils from the rangeland area were significantly contaminated with Cd, moderately contaminated with As, Pb, and Mo, and slightly (low) contaminated with other heavy metals.

For the Dry lake area, the C_d values of the soil samples ranged from 1.36 to 19.33, indicating low to moderate contamination. The percentage contribution of each element to the total C_d in the Dry lake soils was as follows: As (58.41%) > Cd (18.60%) > Pb (5.29%) > Mo (5.0%) > Cr (2.38%) > Zn (2.32%) > Ni (2.10%) > Mn (1.90%) > Cu (1.70%) > Fe (1.31%) > Co (0.99%). Based on C_d values, the soils from the Dry lake area were highly contaminated with As, moderately contaminated with Cd, and slightly (low) contaminated with other heavy metals.

In the agricultural area, the C_d values of the soil samples ranged from 3.74 to 26.96, indicating low to significant contamination. The percentage contribution of each element to the total C_d in the agricultural soils was as follows: Cd (21.63%) > Pb (15.71%) > As (12.48%) > Mo (12.35%) > Zn (7.49%) > Cu (7.32%) > Ni (7.36%) > Cr (6.46%) > Fe (3.95%) > Mn (3.71%) > Co (3.0%). Based on C_d values, the soils from the agricultural area were significantly contaminated with Cd, moderately contaminated with As, Mo, and Pb, and slightly (low) contaminated with other heavy metals.

CONCLUSION

-This study was conducted to evaluate the heavy metal concentrations and contamination levels in various land uses within the Sultan Marshes ecosystem.

-The average concentrations of Cr, Fe, Zn, Co, Cu, and Ni in the soils from the Southern Marshland area; Cd concentrations in the rangeland soils; Pb concentrations in the soils from agricultural areas; and As concentrations in the soils from the Dry lake area were higher than those in other land use types. Some heavy metal concentrations have exceeded historical reference values depending on the different land uses.

-According to C_f values, the soils from the Southern Marshland area showed moderate contamination for Mo, Ni, Cd, Zn, Cr, Pb, and Cu, with low contamination for other heavy metals. In the rangeland area, there was moderate contamination for Cd, As, Mo, and Pb, and low contamination for other heavy metals. In the Dry lake area, there was extreme contamination for As, moderate contamination for Cd, and low contamination for other heavy metals. In the agricultural area, there was moderate contamination for Cd, Pb, As, and Mo, and low contamination for other heavy metals.

-Based on C_d values, soils from the Southern Marshland area exhibited moderate contamination for Mo, Ni, Cd, Zn, Cr, and Pb, with low contamination for other heavy metals. Soils from the rangeland area showed significant contamination for Cd, moderate contamination for As, Pb, and Mo, and low contamination for other heavy metals. Soils from the Dry lake area were extremely contaminated with As, moderately contaminated with Cd, and have low contamination for other heavy metals. Soils from the agricultural area were significantly contaminated with Cd, moderately contaminated with As, Mo, and Pb, and had low contamination for other heavy metals.

-It was generally believed that the heavy metals in the Sultan Marshes ecosystem might originate from natural sources as well as various human activities.

-Measures should be taken to reduce or prevent heavy metal input from human activities in soils under different land uses. Agricultural fertilization and pesticide use, as well as livestock activities, should be controlled, and good agricultural practices should be adopted. Mining activities and mining-based industrial activities in the vicinity must be strictly regulated. Uncontrolled waste and wastewater discharges into the system should be prevented. Specifically, heavy metal pollution sources in Southern Marshland, rangeland, and agricultural areas should be controlled, as there is a higher risk of heavy metals entering the food chain in these areas.

ACKNOWLEDGEMENT

This study was financially supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK). Project number: 122Y062.

REFERENCES

Aksoy, A., 2004. Sultan Sazlığı Flora I., II., III. Ara Raporu ve Final Raporu, Sultan Sazlığı GEF-II Proje Yönetim Birimi, Kayseri.

Aksoy, A., Demirezen, D., Duman, F., 2005. Bioaccumulation, detection and analyses of heavy metal pollution in Sultan Marsh and its environment, J Water Air Soil Pollut., 154: 241-251.

Aksoy, A., Demirezen, D., 2003. Sultan Sazlığı ve Çevresindeki Sucul Ekosistemlerde Ağır Metal Kirliliğinin İncelenmesi, DPT Projesi.

Bai, J., Yang, Z., Cui, B., Gao, H., Ding, Q., 2010. Some heavy metals distribution in wetland soils under different land use types along a typical plateau lake, China, Soil Tillage Res., 106: 344–348.

Demirezen, D., Aksoy, A., 2004. Accumulation of heavy metals in Typha angustifolia (L.) and Potamogeton pectinatus (L.) living in Sultan Marsh (Kayseri, Turkey), Chemosphere, 56: 685–696.

Demirezen, D., Aksoy, A., 2006. Common hydrophytes as bioindicators of iron and manganese pollutions, Ecol Indic., 6:388–393.

DSİ. 1970. Develi Projesi Planlama Raporu, Su-İş Proje ve Mühendislik ve Müşavirlik Bürosu, Ankara.

DSİ. 1995. Kayseri Develi-Yeşilhisar Ovası Revize Hidrojeolojik Etüd Raporu, DSİ XII. Bölge Müdürlüğü, Kayseri, 1-19.

Ferreira, A.C.C., Leite, L.F.C., De Araujo, A.S.F., Eisenhauer, N., 2016. Land-use type effects on soil organic carbon and microbial properties in a semi-arid region of Northeast Brazil, Land Degrad. Dev., 27: 171–178.

Gürer, İ, 2004. Kayseri Sultansazlığı Tabiatı Koruma Alanının Su Kullanım ve Yönetim Planlaması Araştırması, Son Rapor, Çevre ve Orman Bakanlığı, 32-54.

Gürer, İ., Yıldız, E., 2008. Türkiye'nin sulak alan politikalarına genel bir bakış: Sultan Sazlığı Sulak Alanı örneği, TMMOB 2. Su Politikaları Kongresi Bildiri Kitabı s:335-345, 20-22 Mart, Ankara.

Hakanson, L., 1980. An ecological risk index for aquatic pollution control, A sedimentological approach, Water Res., 14: 975-1001.

Jiang, X., Xiong, Z., Liu, H., Liu, G., Liu, W., 2017. Distribution, source identification, and ecological risk assessment of heavy metals in wetland soils of a river–reservoir system, Environ Sci Pollut Res., 24(1): 436– 444.

Karadeniz, N., 1995. Sultansazlığı Örneği'nde Islak Alanların Çevre Koruma Açısından Önemi Üzerinde Bir Araştırma, Ankara Üniversitesi Fen Bilimleri Enstitüsü Peyzaj Mimarlığı Anabilim Dalı Basılmamış Doktora Tezi, Ankara.

Liu, Z., Wang, F., Xie, C., Ning, Z., Li, J., Qin, H., et al., 2015. Assessing heavy metal pollution in surface sediments of saltmarshes in Liaohe Estuary, J Beijing normal univ (nat sci), 54(1): 144–149.

Liu, X., Song, Q., Tang, Y., Li, W., Xu, J, Wu, J., Wang, F., Brookes, P.C., 2013. Human health risk assessment of heavy metals in soil–vegetable system: A multi-medium analysis, Sci Total Environ., 464: 530-540.

Luo, S, Wang, J, Zhou, M, Ye, J, Ke, S, Chen, D, et al., 2018. Spatial distribution and ecological risk assessment of heavy metals in the surface soils of Mangrove Wetland in Donghai Island, Zhanjiang, Ecology Environ Sci.; 27(8): 1547–1555..

Nuralykyzy, B., Wang, P., Deng, X., An, S., Huang, Y., 2021. Heavy Metal Contents and Assessment of Soil Contamination in Different Land-Use Types in the Qaidam Basin, Sustainability, 13: 12020.

Özesmi ,U., Gürer, İ., 2003. Sultan Sazlığı: Biodiversity and natural resources management pilot GEF-II project in Turkey, Journal of IUCN , USA.

Sandilyan, S., Kathiresan, K., 2014. Decline of mangroves - a threat of heavy metal poisoning in Asian, Ocean Coast. Manage., 102:161-168.

Turekian, K.K., Wedepohl, K.H.,1961. Distribution of the elements in some major units of the earth's crust, Geological Society of America Bulletin, 72(2): 175-192.

Türker, O.C., Vymazal, J., 2021. Heavy Metals in Wetlands in Turkey. In: Jawad, L.A. (eds) Southern Iraq's Marshes. Coastal Research Library, Vol 36. Springer, Cham.

Ulusoy, C.K., Yılmaz, M., Erginöz, E., Tursun, U.D., 2003. Develi (Kayseri) Belediyesi, Yeraltısuyu Kullanımı ÇED Ön Araştırma Raporu, İller Bankası Genel Müdürlüğü İçmesuyu Dairesi Başkanlığı, Ankara, 8, 12-19, 33-36, 59-66, 74.

url 1 chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://cdn.standards.iteh.ai/samples/28454/76b916e0bd1f435ba57c1caa0694dadc/ISO-14869-1-2001.pdf

Wang, X., Xu, Y. 2014. Soil heavy metal dynamics and risk assessment under long-term land use and cultivation conversion. Environ Sci Pollut. Res 22: 264-274.

Wang, X., Sun, Y., Li, S., Wang, H., 2019. Spatial distribution and ecological risk assessment of heavy metals in soil from the Raoyanghe Wetland, China, PLOSONE:10, 1-19.

Xia, X. H., Chen, X., Liu, R. M., Liu, H., 2011. Heavy metals in urban soils with various types of land use in Beijing, China, J. Hazard. Mater. 186: 2043–2050.

Xiao, H., Shahab, A., Li, J., Xi, B., Sun, X., He, H.,Yu, G., 2019. Distribution, ecological risk assessment and source identification of heavy metals in surface sediments of Huixian karst wetland ,China .Ecotox.Environ.Safe.,185:109700.

Yalçın, M.G., Battaloğlu, R., İlhan, S., 2007. Heavy metal sources in Sultan Marsh and its neighborhood, Kayseri, Turkey, Environ Geol., 53: 399–415.

Yaşar Korkanç, S., Korkanç, M., Mert, M.H., Geçili, A., Serengil, Y., 2022. Effects of Land Use Change on the Soil Organic Carbon and Selected Soil Properties in the Sultan Marshes, Wetlands, 42: 58.

Zar, H.J., 1996. Biostatistical Analysis, 3rd Edition, Prentice Hall, New Jersey, 662 p.

THE EFFECTS OF MULCHING ON EROSION AND SURFACE RUNOFF IN SOILS GENERATED ON SEDIMENTARY PARENT MATERIAL

Selma YAŞAR KORKANÇ[1], Halil ŞAHİN[2]

[1] Niğde Ömer Halisdemir University, Engineering Faculty, Department of Environmental Engineering, Niğde, Türkiye
[2] Niğde Ömer Halisdemir University, Graduate School of Natural and Applied Sciences, Niğde, Türkiye

Corresponding author e-mail: sykorkanc@ohu.edu.tr

ABSTRACT

Soil and water are among the natural resources that provide crucial services to humans. Water erosion is one of the most significant global problems threatening the sustainable use of these two essential natural resources. To reduce or prevent water erosion, various soil conservation practices are implemented worldwide. Mulching is one such practice used to protect both soil and water. The aim of this study is to determine the effects of different mulch types and application rates on some hydrological (surface runoff, runoff coefficient, time to runoff initiation, percolated and retained water) and erosional (soil loss, sediment concentration) behaviors of soils developed on metamorphic parent material under artificial rainfall conditions in the laboratory. For this purpose, three different mulch types (wheat straw, dry weed, and peanut hay straw) were applied at three different rates (2, 4, 6 tons/ha) to experimental plots created under laboratory conditions. Artificial rainfall was applied at a rate of 97 mm/h for one hour. During the application, the time to runoff initiation was measured, and surface runoff and percolation water were collected to measure the mentioned parameters. The results of the study showed that peanut hay mulch was the most effective application in reducing surface runoff and runoff coefficient. All three mulch types reduced soil loss, with straw mulch being the most effective. The most effective mulch application rate in reducing erosion was found to be 6 tons/ha.

Keywords: Mulching, runoff, rainfall simulation, erosion

INTRODUCTION

Soil is a crucial natural resource due to the services it provides, such as ecosystem functions, its importance in the global biogeochemical cycle, its role in mitigating climate change, and more (Du et al., 2022). Soil erosion is a very serious problem that reduces soil productivity in many parts of the world, especially in semi-arid and semi-humid regions. Soil erosion, driven by excessive surface runoff, leads to the loss of fertile topsoil, resulting in the degradation of soil functions (Biddoccu et al., 2016). The severity and frequency of water erosion are largely dependent on physical conditions. However, the timing and causes of erosion are also strongly influenced by human factors, such as improper land management and changes in land use (Lal, 1984; Cerda, 1994; Montgomery, 2007; Martínez-Casasnovas et al., 2016).

To reduce or prevent erosion and surface runoff, a wide range of methods can be employed in soil and water conservation efforts. Traditional methods primarily include engineering approaches, biological approaches, and agricultural measures (Li et al., 2021). Among agricultural measures, mulching is a method that has been widely used in soil and water conservation worldwide due to its low cost and rapid effectiveness (Fan et al., 2023; Li et al., 2020). Gupta and Gupta (1986) noted that mulching reduces evaporation and increases infiltration, thereby preserving/increasing the soil's moisture content. Mulching can improve the soil's organic matter, nutrient content, and microbiological properties (Jiménez et al., 2016; Srilakshmi et al., 2012). Various studies have reported that mulching reduces runoff, nutrient loss, soil loss, and sediment content (Vega et al., 2012; Lee et al., 2018; Yaşar Korkanç and Şahin, 2021; Yaşar Korkanç and Şahin, 2024; Bogunovic et al., 2023). Organic and inorganic materials can be used as mulch. Organic materials include living cover, straw, plant residues, tree bark, etc. (Li et al., 2021). However, the type of mulch, the amount to be applied, and its cost-effectiveness may vary depending on local conditions. Indeed, Pan et al. (2022) reported a negative relationship between mulch application and soil loss and surface runoff. Therefore, applying different methods under various conditions is deemed necessary to achieve the most effective and feasible conservation.

In this context, the aim of this study is to determine the effects of organic mulch applications, using different types and doses of mulch, on certain hydrological (surface runoff, runoff coefficient, time to runoff initiation, percolation water, and water remaining in the soil) and erosional (soil loss, sediment concentration) behaviors of the soil.

MATERIAL AND METHOD

In this study, the topsoil (0-20 cm) developed on sedimentary bedrock within the boundaries of Gümüşler Town in Niğde province was used to establish the experimental plots. The land from which the soil samples were taken is flat to nearly flat with a slight undulation. The current land use is pasture (but previous land use was farmland). The soil samples have a sandy loam texture, with 90.38% sand, 3.55% clay, 6.07% silt, a pH of 7.02, electrical conductivity of 125.15 μS/cm, loss on ignition of 5.35%, and aggregate stability of 4.95%. The study utilized an artificial rainfall simulator as detailed in Yaşar Korkanç (2018). The artificial rainfall was applied at a rate of 97 mm/hour for 1 hour using a Lechler 460.646-type nozzle under a pressure of 0.5 bar. The plots where the soil samples were placed measured 30x50x15 cm and were made of metal. The erosion plots had drainage holes at the bottom, where gauze was first laid, followed by a 7 cm layer of sand, which was leveled. Another layer of gauze was placed on top of the sand, and then the plot was filled with 5 cm of soil, which had been passed through an 8 mm sieve, up to the surface runoff pipe exit, and the surface was carefully leveled (Akalan, 1967; Taysun, 1986). The experimental plots were treated with three different mulch materials (mixed grass, wheat straw, and peanut plant residues) applied at three different doses (Dose 1: 2 tons/ha, Dose 2: 4 tons/ha, and Dose 3: 6 tons/ha) (Figure 1). The materials used as mulch were dried at 65°C before application. Immediately after the mulch application, the experimental plot was placed on a stand with a 9% slope, and artificial rainfall was applied (Yaşar Korkanç and Şahin, 2021). The study was conducted using a two-factor (mulch type and mulch dose) completely randomized block design. During the artificial rainfall application, the time of runoff initiation was measured, and the surface runoff and percolation water were collected for calculating parameters such as surface runoff, runoff coefficient, soil loss, sediment concentration, percolation water, and water remaining in the soil(retained water). Statistical evaluations were conducted using the SPSS 24.0 software package. The effects of different types and doses of mulch on the mentioned parameters were assessed using variance analysis (ANOVA) (P=0.05), and if differences were found, the differences between the means were determined using the Duncan test (Zar, 1996).

Figure 1. A view of the experimental plots used in the study

RESULTS AND DISCUSSION

The statistical evaluation results showed that different types and doses of mulch applications significantly affected the parameters of surface runoff, runoff coefficient, time to runoff initiation, percolation water, retained water, soil loss, and sediment concentration (P<0.05) (Figures 2-8)

Surface Runoff

The change in surface runoff observed after the application of different mulch types was presented in Figure 2. Compared to the control plot, peanut hay mulch was found to be more effective in reducing surface runoff than the other mulch types. In contrast, an increase in surface runoff was observed in the plots where the other two types of mulch were applied (Figure 2)

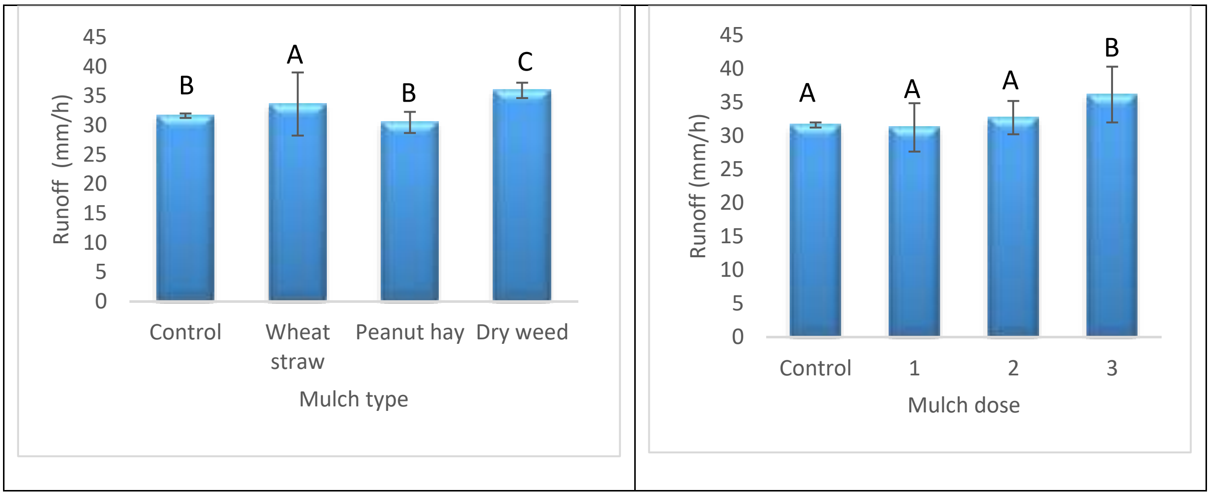


Figure 2. Changes in surface runoff according to different types and doses of mulch applications

The changes in surface runoff according to different mulch doses were shown in Figure 2. The surface runoff from the plots where the 1st dose was applied close to those of the control plot. As the dose application amount increased, a general increase in surface runoff was observed. The highest increase compared to the control plot was noted in the plots where the 3rd dose was applied (Figure 2).

Runoff Cofficient

Figure 3 shown the change in runoff coefficient according to different mulch types. The runoff coefficient was most reduced in the plots where peanut hay mulch was applied compared to the control plot. In contrast, straw and dry weed mulch were found to increase the runoff coefficient (Figure 3).

The change in runoff coefficient in plots with different dose applications was presented in Figure 3. The values closest to the runoff coefficient of the control plot were found in the plots where the first dose was applied. Overall, an increase in mulch dose resulted in a higher runoff coefficient (except 1^{st} dose) (Figure 3).

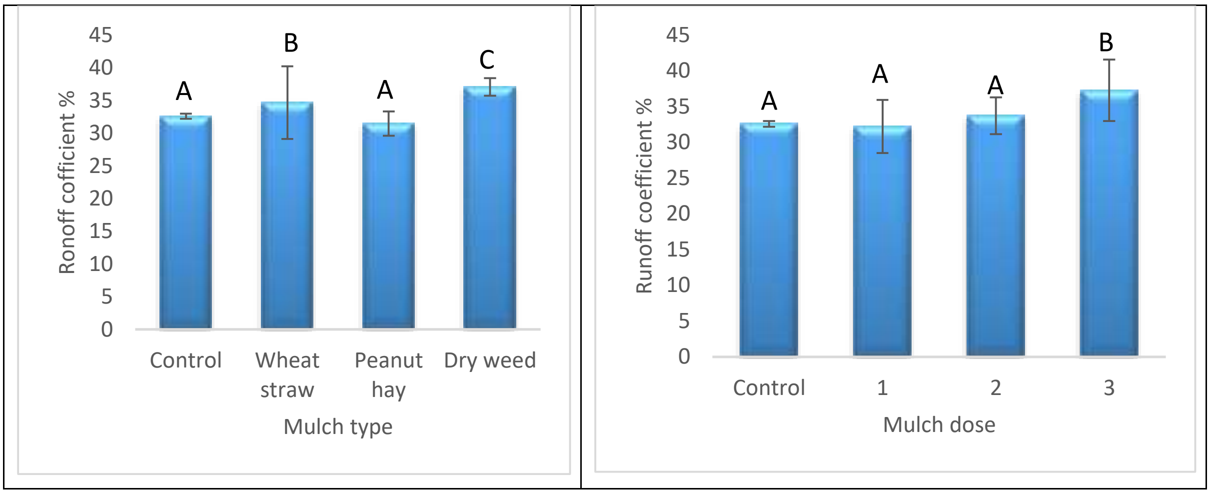


Figure 3. Changes in runoff coefficient according to different types and doses of mulch applications

Time to Runoff Initiation

The change in time to runoff initiation according to different mulch types was shown in Figure 4. All three mulch types led to a decrease in time to runoff initiation compared to the control plot (Figure 4).

The change in time to runoff initiation with different mulch doses was presented in Figure 4. All three doses reduced the time to runoff initiation compared to the control. However, as the dose amount increased, a tendency for time to runoff initiation to increase was observed (Figure 4).

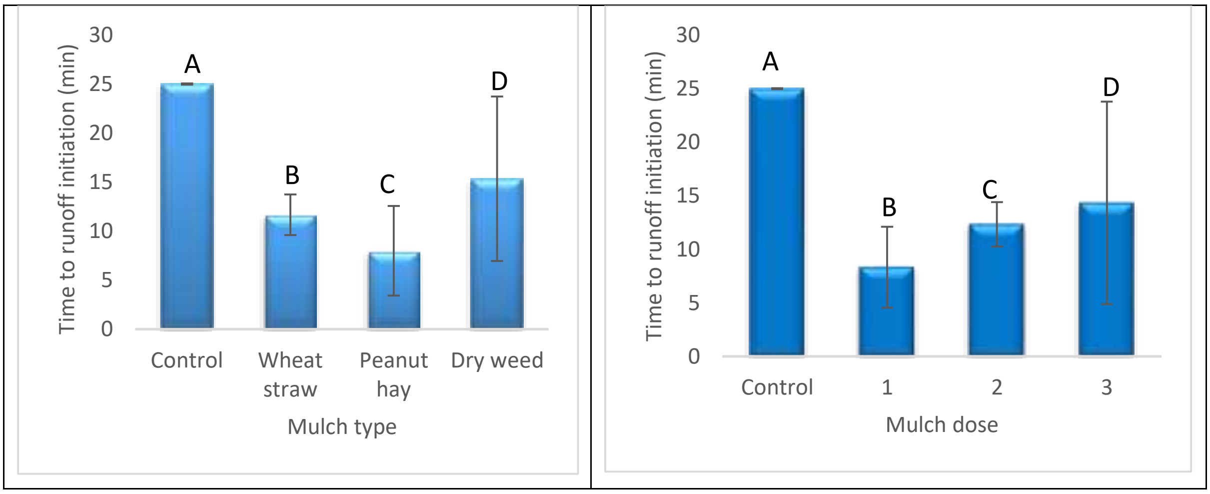


Figure 4. Changes in time to runoff initiation according to different types and doses of mulch applications

Percolated (Drained) Water

The change in the amount of percolation water according to different mulch types was presented in Figure 5. All three mulch types caused a decrease in the amount of water percolated from the soil. The mulch type with the highest decrease was in the plots where straw mulch was applied, followed by dry weed mulch. The values closest to the percolation water in the control plots were found in the peanut hay mulch applications (Figure 5).

The changes in percolation water according to different mulch doses were presented in Figure 5. All three mulch dose applications caused a decrease in percolation water. In general, it was observed that percolation water tended to increase as the mulch dose increased (Figure 5).

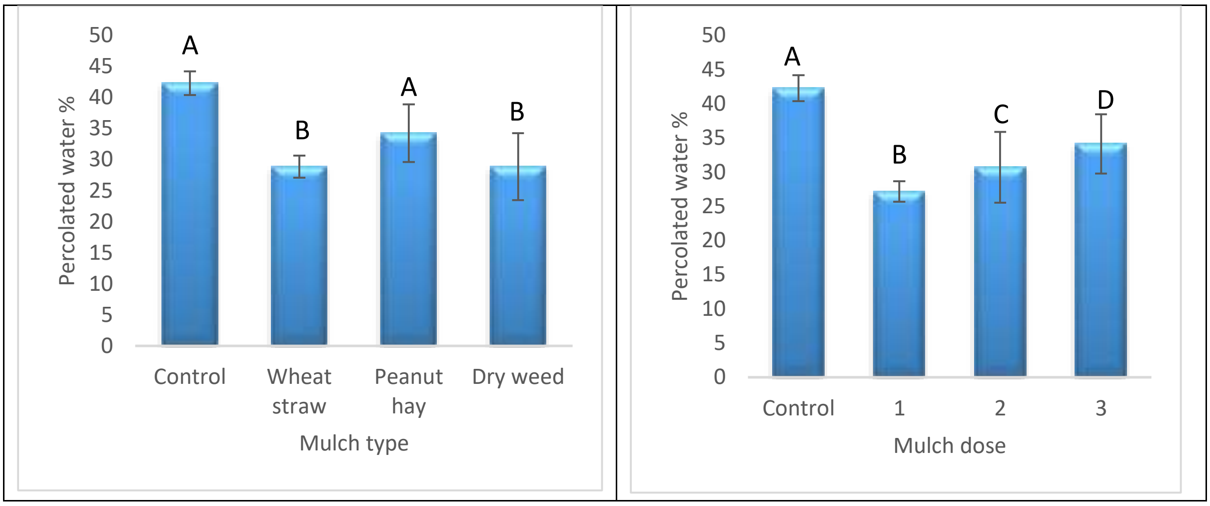


Figure 5. Changes in percolated water according to different types and doses of mulch applications

Retained Water

The changes in retained water in the soil according to different mulch types were presented in Figure 6. It was found that all three mulch types increased the water retention in the soil. The most effective mulch type in increasing the retained water in the soil was straw mulch. The least effective mulch type was determined to be dry weed mulch (Figure 6).

The variation in retained water in the soil according to different dose applications was as shown in Figure 6. All three mulch doses had a positive effect on water retention in the soil. The most effective mulch dose is the 1st dose, while the least effective mulch dose is the 3rd dose application (Figure 6).

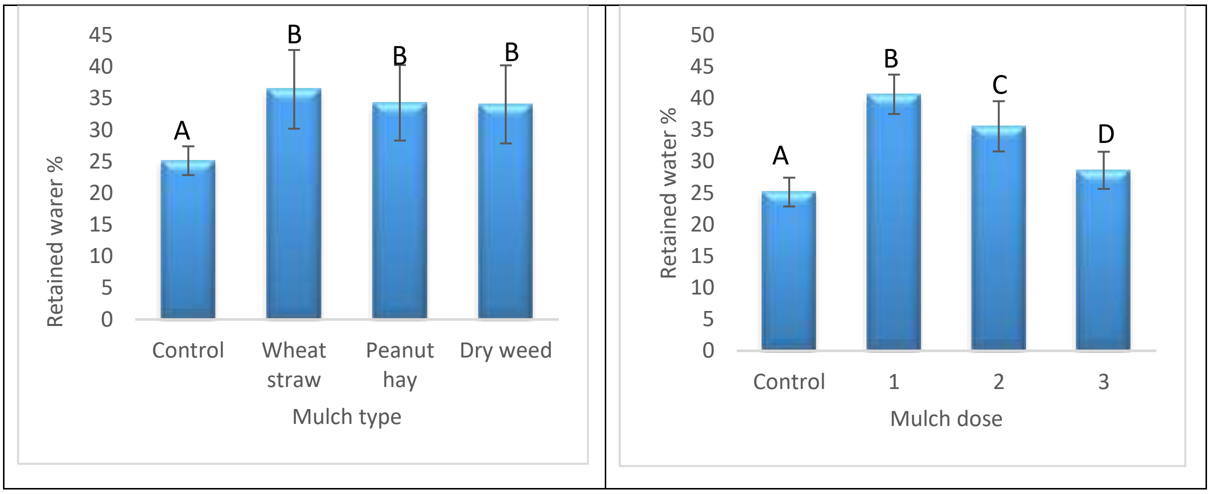


Figure 6. Changes in retained water according to different types and doses of mulch applications

Soil Loss (Erosion)

The changes in soil loss due to different types of mulch applications was presented in Figure 7. It was found that all three types of mulch were effective in reducing soil loss. The most effective mulch type for preventing soil loss was wheat straw mulch, followed by peanut hay mulch and dry weed mulch (Figure 7).

The variation in soil loss according to different mulch doses was shown in Figure 7. All mulch doses reduced soil loss compared to the control values. The most effective mulch dose for preventing soil loss was the 3rd dose application, followed by the 1st and 2nd dose applications (Figure 7).

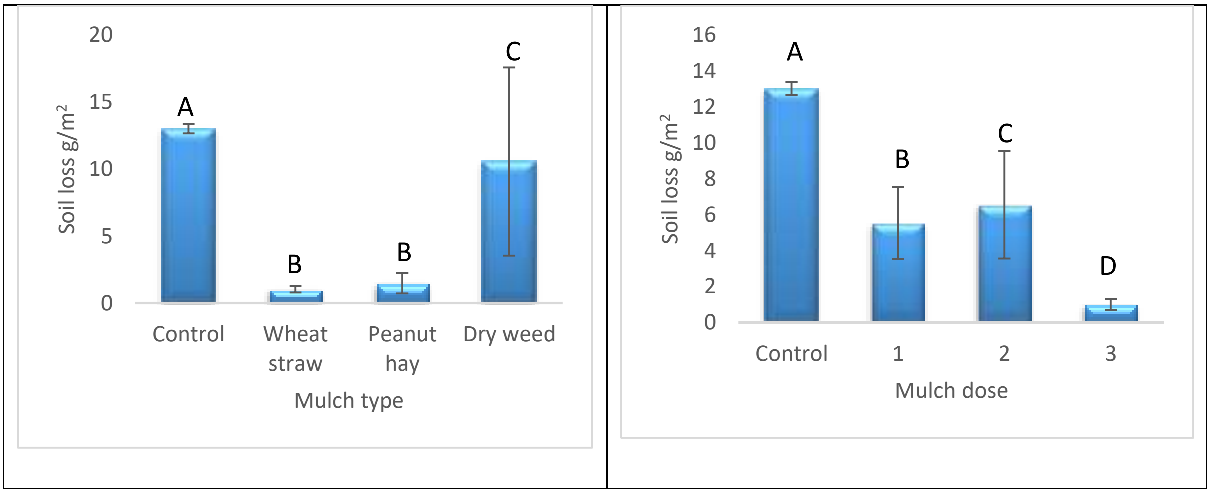


Figure 7. Changes in soil loss according to different types and doses of mulch applications

Sediment Concentration

The variation in sediment concentration according to different types of mulch was shown in Figure 8. Sediment concentration decreased in plots with wheat straw mulch and peanut hay mulch compared to the control, while it did not decrease in plots with dry weed mulch. The most effective mulch type for reducing sediment concentration was wheat straw mulch (Figure 8).

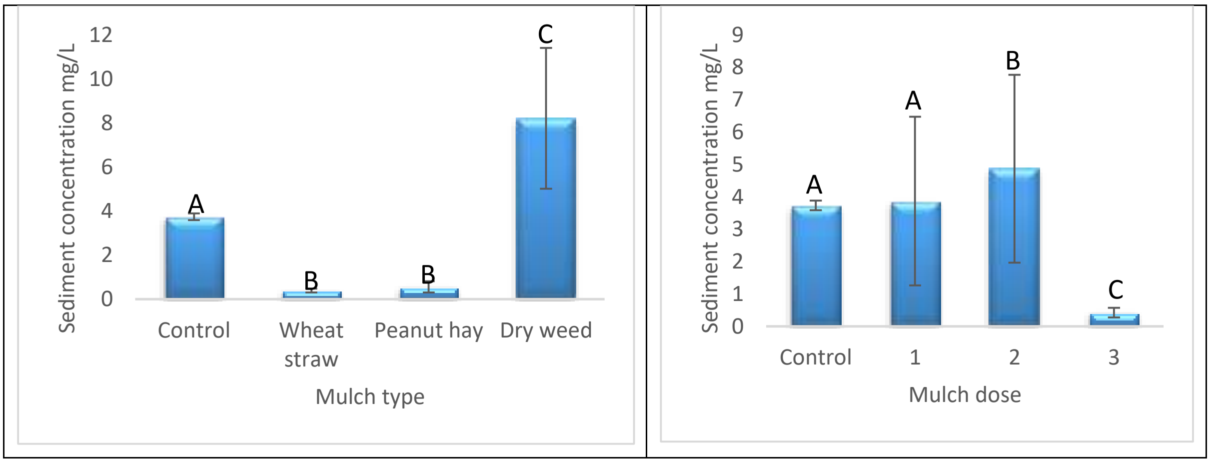


Figure 8. Changes in sediment concentration according to different types and doses of mulch applications

CONCLUSIONS

This study was conducted to investigate the effects of different types and amounts of organic mulch on various hydrological and erosional behaviors of soils. The most effective mulch type and dose for reducing runoff and runoff coefficient wasthe 1st dose of wheat straw mulch. All three types and doses of mulch caused a reduction in the time to runoff. As the mulch dose increased, the time to runoff initiation also increased. All three types and doses of mulch resulted in a decrease in percolation water. Each type and dose of mulch was effective in increasing the amount of water retained in the soil, with straw mulch being the most effective type and the 1st dose application being the most effective dose. All three mulch types were effective in reducing soil loss, with wheat straw mulch being the most effective type and the 3rd dose application being the most effective dose. Based on the results obtained under these conditions, mulching can be recommended as a nature-based practice for reducing soil loss and conserving soil moisture. However, to determine the most economically suitable type and dose of mulch, the number of studies in different conditions should be increased with regional trials.

ACKNOWLEDGEMENT

The authors would like to thank Prof. Dr. Mustafa KORKANÇ from Department of Geological Engineering, for his laboratory support during the plot trials of this study.

REFERENCES

Akalan, İ., 1967. Toprak Fiziksel Özellikleri ve Erozyon. Ankara Üniversitesi Ziraat Fakültesi Yıllığı, (3-4):490-503.

Bogunovic, I., Hrelja, I., Kisic, I., Dugan, I., Krevh, V., Defterdarovic, J., Filipovic, V., Filipovic, L., Pereira, P., 2023. Straw mulch effect on soil and water loss in different growth phases of maize sown on stagnosols in Croatia. Land, 12: 765.

Biddoccu, M, Ferraris, S, Opsi, F., Cavallo, E., 2016. Long-term monitoring of soil management effects on runoff and soil erosion in sloping vineyards in Alto Monferrato (North–West Italy), Soil Till Res, 155: 176-189.

Cerdà, A., 1994. The response of abandoned terraces to simulated rain. In: Rickson, R.J. (Ed.), Conserving Soil Resources: European Perspective. CAB International, Wallingford, 44–55.

Du, X., Jian, J., Du, C., Ryan, D., 2022. Stewart,Conservation management decreases surface runoff and soil erosion,International Soil and Water Conservation Research,10 (2): 188-196.

Fan, D., Jia, G., , Wang, Y., Yu, X., 2023. The effectiveness of mulching practices on water erosion control: A global meta-analysis, Geoderma, 438:116643.

Gupta, J.P., Gupta, G.K., 1986. Effect of tillage and mulching on soil environment and cowpea seedling growth under arid conditions. Soil Till Res. 7: 233–240.

Jiménez, M.N., Fernández-Ondoño, E., Ripoll, M.Á., Castro-Rodríguez, J., Huntsinger L., Navarro, F.B., 2016. Stones and organic mulches improve the Quercus ilex L. afforestation success under Mediterranean climatic conditions. Land Degrad Dev. 27: 357–365.

Lal, R., 1984. Mulch requirements for erosion control with the no-till system in the tropics: a review. Challenges in African Hydrology and Water Resources. Proceedings of the Harare Symposium. IAHS Publ. No. 144

Lee, G., Mclaughlin, R.A., Whitely, K.D., Brown, V.K., 2018. Evaluation of seven mulch treatments for erosion control and vegetation establishment on steep slopes. J Soil Water Conserv. 73:434–442.

Li, R., Li, Q., Pan, L., 2021. Review of organic mulching effects on soil and water loss.Archives of Agronomy and Soil Science, 67(1): 136–151.

Martínez-Casasnovas, J.A., Ramos, M.C., Benites, G., 2016. Soil and water assessment tool soil loss simulation at the sub-basin scale in the Alt Penedès-Anoia vineyard region (Ne Spain) in the 2000s. Land Degrad. Dev. 27 (2):160–170.

Montgomery, D.R., 2007. Soil erosion and agricultural sustainability. PNAS 104: 13268–13272.

Pan, L., Li, Q., Gao, J., Yang, P., Huang, K., Zhang, L., Li, R., 2022. Study on appropriate straw mulching application rate on sloping farmland in karst area of Southwest China based on soil and water conservation function. Archives of Agronomy and Soil Science, 68(14): 2027–2044.

Srilakshmi, A, Dakshayani, R, Saigopal, DVR, Narasimha, G., 2012. Influence of forest litter on soil enzyme activities. Ecol, Environ Conserv. 18:105–112.

Taysun, A.1986. Gediz Havzasında Rendzina Tarım Topraklarında Yapay Yağmurlayıcı Yardımıyla Taşlar, Bitki Artıkları ve Polivinilalkolün (PVA) Toprak Özellikleri ile Birlikte Erozyona Etkileri Üzerine Araştırmalar, Ege Üniversitesi Ziraat Fakültesi, Yayın No: 474.

Vega, J.A., Fernández, C., Fonturbel, T., 2015. Comparing the effectiveness of seeding and mulching+seeding in reducing soil erosion after a high severity fire in Galicia (NW Spain). Ecol Eng. 74:206–212.

Yaşar Korkanç, S., 2018.Effects of the land use/cover on the surface runoff and soil loss in the Niğde Akkaya Dam Watershed, Turkey, Catena, 163: 233–243.

Yaşar Korkanç, S., Şahin, H., 2024. The efficiency of different mulch types and dose applications on runoff, soil, and nutrient losses under simulated rainfall. Land Degradation & Development, 35(14): 4201–4212.

Yaşar Korkanç, S., Şahin, H., 2021. The effects of mulching with organic materials on the soil nutrient and carbon transport by runoff under simulated rainfall conditions. Journal of African Earth Sciences, 176, 104152.

Zar, H.J., 1996. Biostatistical Analysis, 3rd Edition, Prentice Hall, New Jersey, 662 p.

ABRUPT SHIFTS IN ADRIATIC SEA FISH POPULATIONS

Elvis KAMBERI[12], Edmond HALA[1], Alexandra M. BLÖCKER[2], Marco KULE [1,3], Christian MÖLLMANN[2]*

[1] *Department of Aquaculture and Fisheries, Agricultural University of Tirana, Albania*
[2] *Institute for Marine Ecosystem and Fisheries Science (IFM), Center for Earth System Research and Sustainability (CEN), University of Hamburg, Germany*
[3] *Directory of Fisheries and Aquaculture and Policies and Programmes, Ministry of Agriculture and Rural Development, Albania*

**Corresponding author e-mail: ekamberi@ubt.edu.al*

ABSTRACT

Management and sustainable use of fish stocks is considered to be a challenging task due to the continuous interaction of internal and external drivers. These forces can move the stocks out of their equilibrium state, which can result in abrupt shifts. Identifying possible causes of such changes in natural systems remains difficult, as they can arise under various circumstances. Threats are often not recognized until the species have suffered large population declines. Abrupt changes may hinder the success of management measures and have severe ecological, economic and social consequences. In this study we used a systematic approach that classifies a time series to a trajectory type (no-change, linear, quadratic and abrupt change) along with statistical changepoint analyses to identify past abrupt changes in the biomass of the most important commercial fish species in the Adriatic Sea. This classification approach allowed us to identify the best-fitted trajectory and also investigate additional change points in the biomass. Our analysis shows that an abrupt trajectory better explains the dynamics of five out of the seven species we investigated, compared to the other trajectories considered. This study represents the first step towards the identification of non-linear dynamics in the biomass of Adriatic Sea fish species and evaluating their potential drivers. Considering the magnitude and rapid change of many drivers during the Anthropocene, diagnosing abrupt changes and their underlying causes becomes particularly important, due to the profound and increasing consequences for the sustainability of fish stocks.

Keywords: abrupt change, fish stocks, biomass, non-linear dynamics

INTRODUCTION

Over the last decades, despite the efforts of fisheries management to improve the status of many fish stocks by rebuilding their populations to sustainable levels, nearly 50 % of the stocks still remain below biomass targets (Britten et al., 2021). Despite recovery efforts, the sustainable use of fish stocks can be hindered or inhibited by the continuous interactions of multiple external drivers such as fishing and climate change (Brander, 2007; Lotze et al., 2011b;

Conversi et al., 2015). Although it is generally assumed that natural populations respond in a predictable and gradual manner to these external disturbances, such observations are rarely encountered (Clark and Luis, 2020). Instead, several studies have shown sudden and unexpected abrupt shifts of populations (Folke et al., 2004; Ratajczak et al., 2018; Turner et al., 2020). Abrupt changes are difficult to predict and can occur under various circumstances, including both gradual and sudden changes in drivers, as well as interactions among multiple drivers (Bestelmeyer et al., 2011; Ratajczak et al., 2018).

Abrupt changes differ widely in scale, some are mild and can be reversed by restoring the driver variables to previous levels, whereas in other cases they can persist long after the drivers returned to their initial conditions (Bestelmeyer et al., 2011; Ratajczak et al., 2018). Often, they are considered indicators of transitions between alternative states. Abrupt shifts represent a critical transition whose likelihood increases as the system approaches a so-called tipping point, where even a small disturbance can initiate a self-propagating shift to a new state (Scheffer, 2009).

Depending on their characteristics, some ecosystems may be able to absorb stronger disturbances than others, an ability recognized as resilience. However, marine ecosystems are generally recognized to be particularly vulnerable to drastic and unexpected changes (deYoung et al., 2008). The likelihood of these abrupt changes is expected to increase in both strength and frequency in the future following the intensification of anthropogenic disturbances and climate change (Möllmann et al., 2015; Turner et al., 2020; Clark and Luis, 2020). These changes can have important socio-ecological consequences, particularly under the increasing pressure from climate driven disturbances (O'Leary et al., 2017; Turner et al., 2020). Even though numerous examples of non-linear dynamics in various marine systems world-wide led to severe ecological, economic and social consequences, current management strategies still rely largely on continuous dynamics (Levin and Möllmann, 2015; Sguotti and Cormon, 2018).

The Adriatic Sea is considered as one of the most impacted areas in the Mediterranean due to anthropogenic activities, with fishing and land-based pollution identified as the major contributors (Coll et al., 2012; Micheli et al., 2013). The basin is considered as the most productive area across the Mediterranean and a hotspot for biodiversity, hosting more than 7000 species (Furlan et al., 2019). It has a long history of exploitation which has caused significant changes in the species composition with dramatic declines of top predators, demersal and pelagic species (Lotze et al., 2011a; Piroddi et al., 2017; Sguotti et al., 2022). High fishing pressure over the years has caused significant reductions in the biomass of the most important fish stocks and management has failed to reduce fishing pressure to the required target levels (Piroddi et al., 2017). In addition, recent climate change projections have identified the Adriatic as one of the most vulnerable areas of the world ocean (Coll et al., 2012). Climate change is expected to affect its fisheries as well as biodiversity and ecosystem functioning in general (Marbà et al., 2015; Piroddi et al., 2017; Hidalgo et al., 2019b). Recent studies have identified fisheries and climate change as the main drivers of past regime shifts in fish community composition, with previously dominant large-bodied species being replaced by smaller, lower trophic level organisms (Sguotti et al., 2022).

Our analysis revealed that an abrupt trajectory better explains the dynamics of five out of seven species we studied. This study marks the first step toward identifying non-linear dynamics in the biomass of Adriatic Sea fish species. Considering the magnitude and rapid change of many drivers during the Anthropocene (Rockström et al., 2009), diagnosing abrupt changes and their underlying causes becomes particularly important due to the profound and increasing ecological, social and economic losses and to ensure an effective management of fish stocks.

MATERIALS AND METHODS

Data

Our analyses of trajectory selection and change point identification were based on stock assessment data for seven commercially important fish species from the Adriatic Sea: European hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*), common sole (*Solea solea*), Norway lobster (*Nephrops norvegicus*), deep water rose shrimp (*Parapenaeus longirostris*), sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*). Data for European hake, red mullet, common sole and deep water rose shrimp were obtained from the Joint Research Center (JRC) publications repository (JRC, 2023). Data for Norway lobster, sardine and anchovy were obtained from the General Fisheries Commission for Mediterranean (GFCM) database of stock assessment results (https://www.fao.org/gfcm/data/star/en/). The longest time series corresponded to common sole, spanning 62 years (1962-2020), while the shortest time series were for sardine and anchovy, covering 2000 to 2023. Data for hake included the years 1998 – 2022, for red mullet 1973-2021, for Norway lobster 1972-2021 and deep water rose shrimp 1996-2022. The data included information on recruitment (R), spawning stock biomass (SSB), landings and fishing mortality (F). The only exception was the deep water rose shrimp, for which SSB data were unavailable because the stock assessment model used a production model (SPICT) and not a fully analytical one (JRC, 2023), as a consequence total biomass (B) data was used instead.

Statistical analyses

In order to determine the presence of abrupt shifts in spawning stock biomass of the most important commercial species of the Adriatic Sea, we followed a two-step process combining trajectory classification and changepoint analysis. By following the approach by Pélissié et al. (2024) we employed a trajectory classification methodology to evaluate the presence of abrupt shifts in our time series. This approach integrates several existing methods in a three-step classification process, including model selection, validation and reliability confirmation, in order to classify any time series to one of the following trajectory types, i.e. linear, non-linear (quadratic) or abrupt change, and confirms the occurrence of potential abrupt shifts. The most parsimonious model was selected by comparing Akaike Information Criteria values corrected for small sample size (AICc). The reliability of the classification was assessed using three different indices: weighted AIC (wAIC), Leave One Out (LOO) and Normalized Root Mean Square Error (NRMSE). Considering that the classification is more reliable for timeseries of at least 25 timepoints and in order to minimize the risk of missing abrupt shifts, the breakpoint validation step was skipped for the shorter time series corresponding to hake, deep water rose shrimp, anchovy and sardine (see Pélissié et al., 2024). This step served as a primary filter to identify abrupt trajectories in the spawning stock biomass (biomass in the case of deep water rose shrimp) of the analysed species. The species, whose trajectory was not classified as abrupt were not considered for further analyses.

In addition, after identifying the trajectory type, abrupt changes in the time series of SSB for each species selected in the first step were detected using two different statistical change point methods (Möllmann et al., 2021; Blöcker et al., 2023), each employing a different algorithm, provided by the R packages *changepoin*t (Killick and Eckley, 2014) and *bcp* (Erdman and Emerson, 2008). These methods identify the points where changes are observed in the statistical properties of a time series. Within the *cpt.mean* function of the *changepoint* package we used the *BinSeg* algorithm and set a minimum segment length of at least 5 years between change points to ensure a reasonable length for quasi-stable periods. In contrast, the Bayesian change point approach, applied through the package *bcp*, calculates the posterior probabilities of change at any given point in time series.

Table 1. Trajectory classification and abrupt changes in Adriatic Sea fish stocks

Species	**Trajectory Classification**		**1st change point**		**2nd change point**	
	Trajectory	Break date	BinSeg	BCP	BinSeg	BCP
European hake	Abrupt	2009	2009	2010	2017	2019
Red mullet	Abrupt	1992	1992	1992	2011	2012
Common sole	Abrupt	2003	2003	2003	2014	2014
Deep water rose shrimp	Abrupt	2015	2015	2015	-	-
Anchovy	Abrupt	2010	2010	2010	-	-
Sardine	Quadratic	-	-	-	-	-
Norway lobster	Linear	-	-	-	-	-

RESULTS AND DISCUSSION

In our analyses, we combined a systematic approach for trajectory classification introduced by Pélissié et al., (2024) with changepoint analyses to investigate abrupt shifts in biological data-series from the Adriatic Sea. The systematic approach allowed us to classify the time series to a trajectory type: no change, linear, quadratic and abrupt change. Meanwhile, the statistical changepoint analysis, employing two different methods, identified the location of additional past abrupt changes in the biomass (SSB and B) of the most important commercial fish species in the Adriatic Sea; European hake, red mullet, common sole, Norway lobster, deep water rose shrimp, sardine and anchovy. The classification process identified abrupt trajectories for five out of 7 analysed species: European hake, red mullet, common sole, deep water rose shrimp and anchovy (Table 1). For the remaining species, a linear trajectory for Norway lobster and a quadratic trajectory for sardine were identified. Hence, these two species were excluded from further identification of abrupt changes location through changepoint analyses. Due to the method's sensitivity to the length of the time series and to minimize the risk of missing abrupt shifts (Pélissié et al., 2024), only the first step was applied to the data for European hake (25 timepoints), deep water rose shrimp (27 timepoints), sardine (24 timepoints) and anchovy (24 timepoints). This adjustment resulted in an abrupt trajectory classification for European hake and anchovy, which were previously classified as quadratic and linear, respectively. However, it did not identify a different trajectory for deep water rose shrimp and sardine.

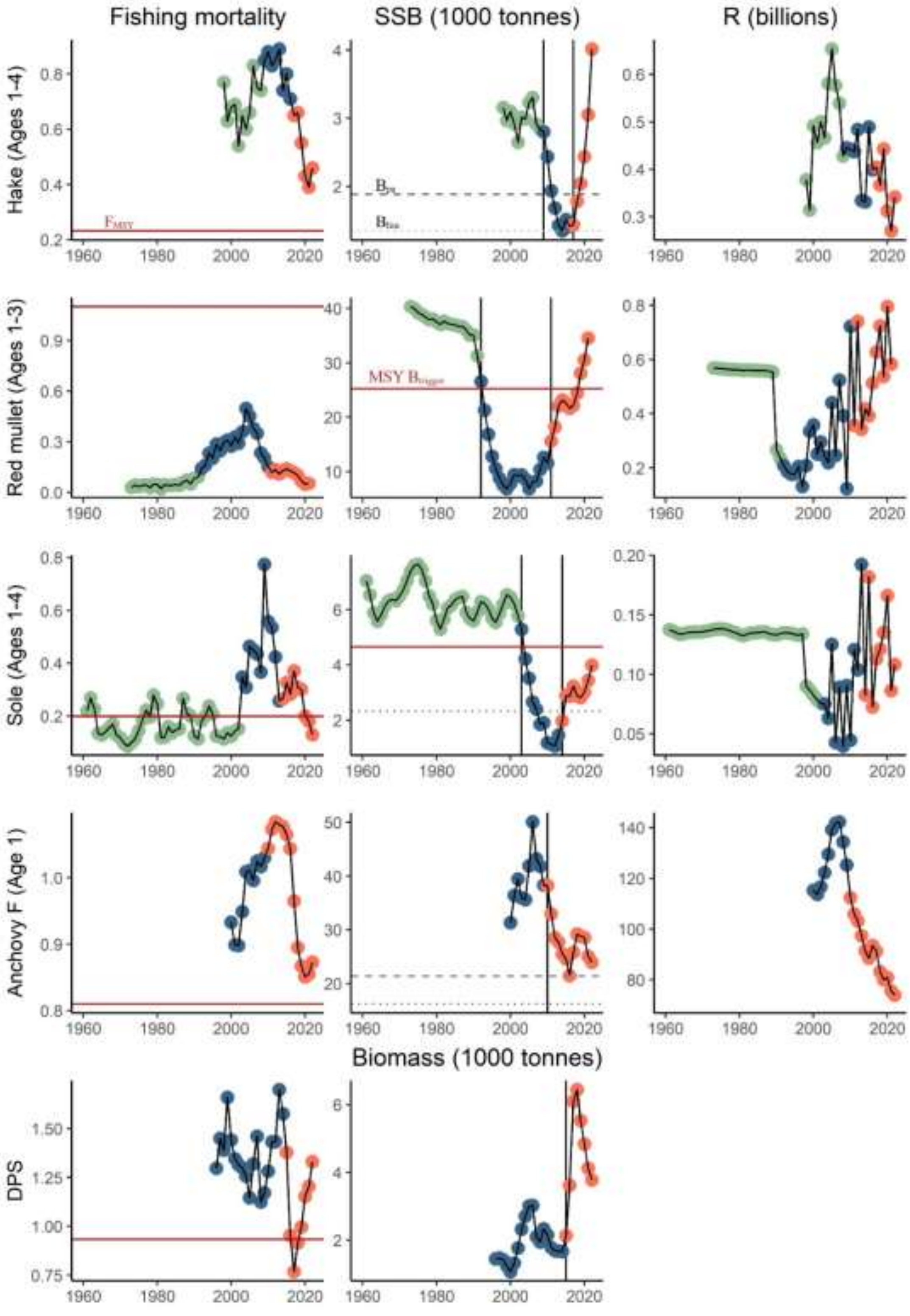


Figure 1. Abrupt changes in the spawning stock biomass (SSB) and total biomass (B) of Adriatic Sea fish stocks. Color ranges indicate the identified periods for SSB or B. The figure includes time series of fishing mortality (F), SSB or B, and recruitment (R). Horizontal lines indicate the management reference points: in the first column, the F level that leads to the Maximum Sustainable Yield (F_{MSY}); in the second column, the SSB level triggering

management actions (MSY $B_{trigger}$) as well as the precautionary level (B_{pa}) and limit reference point of SSB (B_{lim}); DPS: deep water rose shrimp (*Parapenaeus longirostris*).

The analysis of the time series of fishing mortality (F), spawning stock biomass (SSB) and recruitment revealed important insights in the development of the targeted fish stocks. Fisheries management succeeded to reduce F in the mid-2010s decade, interrupting its previous increasing trend (JRC, 2023). For three out of five analysed stocks (European hake, anchovy and deep water rose shrimp) F was above the present F_{MSY} target (Figure 1 – left column).
Management measures adopted in the Mediterranean fishery rely on controlling fishing effort and in the implementation of technical measures, such as mesh size regulation, minimum landing size and the closure of areas and seasons for fishing (Colloca et al., 2013; Vasilakopoulos et al., 2014; Cardinale et al., 2017). However, the management approach limited mainly on input control, has been found to be ineffective due to a lack of adherence to scientific evidence and the absence of strong institutional frameworks (Cardinale et al., 2017). Regardless of the species, the 2000s decade marks an important increase of F which however decreased during the last decade. Still, the target F_{MSY} is not reached for all the stocks. Here we showed that, with the exception of red mullet, where fishing mortality has constantly remained below $F_{MSY,}$ and common sole, where management efforts successfully reduced F below target values only during the last two years, the exploitation rate for the remaining species (hake, deep water rose shrimp and anchovy) is significantly higher than the current estimated F_{MSY}. Moreover, the realized effort reductions have always been much smaller than advised and have not always been followed by a corresponding reduction in fishing mortality (Colloca et al., 2013; Cardinale et al., 2017).

Effort control had varying effects on the dynamics of different Adriatic stocks. All stocks experienced a drastic decrease in SSB to levels far below sustainable thresholds following an increase in F. While the reduction of F resulted in a significant increase in the SSB of hake, red mullet, common sole and the total biomass of deep water rose shrimp, the opposite was observed for anchovy, where SSB and R continued their negative trend (Figure 1- middle and right column). Although the drastic decrease in anchovy SSB is at least partially related to heavy commercial exploitation (Santojanni et al., 2006), with F reaching values up to 30% higher than the target F_{MSY} (JRC, 2023), the lack of recovery of SSB, even after reducing F, suggests that stock dynamics are also influenced by additional factors. Furthermore, the trend in R may also reflect stock status, as R tends to follow SSB patterns in overexploited stocks (Szuwalski et al., 2015), such as in the case of anchovy (GFCM, 2024).
Previous studies have shown that anchovy recruitment is highly sensitive to environmental conditions such as sea surface temperature, chlorophyll-a, wind mixing and river runoff. These drivers influence directly and indirectly the physiology, growth and behaviour of the species, as well as ecosystem productivity (Martín et al., 2012; Santojanni et al., 2006). The Adriatic Sea has experienced a continuous increase in sea surface temperatures (Bonacci and Vrsalović, 2022) in recent years, which may be contributing to the ongoing decline in anchovy stock (Martín et al., 2012). Although anchovy is generally expected to positively correlate with rising temperatures (Tzanatos et al., 2014), findings in the Western Mediterranean indicate that their

productivity is negatively affected by higher-than-average temperatures due to these combined influences (Santojanni et al., 2006; Martín et al., 2012).
While recruitment levels of red mullet and common sole showed an increasing trend, a decline in R is also observed in the European hake stock, regardless of SSB development (Figure 1-right column). This decline can be attributed to several factors, including fishing and environmental variability (Colloca et al., 2013; Vasilakopoulos et al., 2014; Hidalgo et al., 2019a). Throughout the Mediterranean, hake fisheries are characterized by low selectivity and the overexploitation of recruits and juveniles, as larger individuals are less susceptible to the trawling due to offshore occurrence of the spawners (Colloca et al., 2013; Vasilakopoulos et al., 2014). Additionally, the survival of hake juveniles is strongly influenced by environmental factors, particularly by primary production. In the Adriatic, hake survival is negatively correlated with primary production and food availability, likely due to the increased inter-specific competition within the nektobenthic community, which may limit food availability for hake and reduce juvenile survival (Hidalgo et al., 2019a). Even though these exploitation patterns are associated with reducing the risks of stock depletion and collapse, their effects on yield, population structure and economic consequences remain poorly investigated (Colloca et al., 2013).
A similar discussion applies to the common sole stock. Although management measures have successfully reduced F over the last decade, leading to an increasing trend in SSB and R, SSB remains below the target B_{MSY} values and R has exhibited considerable fluctuations during this period (Figure 1). The species displays distinct spatial distribution patterns, with juveniles found closer to the shore and mature individuals inhabiting offshore areas where trawl operations are challenging. Additionally, the distribution of fishing effort shows a high overlap with juvenile distribution areas, indicating significant juvenile mortality that determine spawning stock biomass trend and recruitment rates (Scarcella et al., 2014).

Furthermore, statistical changepoint analyses revealed several abrupt changes in the SSB and B. For hake, red mullet and common sole, two abrupt changes were detected, indicating three distinct periods (Table 1). In all cases, the first abrupt change was detected from the *chngpt* algorithm incorporated in the trajectory identification process, as well as the *BinSeg* and *bcp* algorithms. The second abrupt change was detected only by the *BinSeg* and *bcp* algorithms.
The low SSB phases of these species, defined by the two abrupt changes, persisted across different decades. The earliest abrupt change was observed in the red mullet SSB at the beginning of the 1990's (1992). This low phase lasted approximately 20 years before a second abrupt change occurred in 2011. In addition, common sole and hake experienced similar changes in the subsequent decades. Common sole experienced its first abrupt change at the beginning of the 2000's (2003), with SSB remaining at low levels until the second abrupt change in 2014. Hake experienced these changes later, with a low SSB period persisting during the 2010s (2009-2017). Meanwhile, only one changepoint was identified for the remaining species: anchovy in 2010 and deep water rose shrimp in 2015.
Abrupt changes can be considered as an initial indicator of regime shifts (Blöcker et al., 2023), however they do not necessarily indicate a transition to an alternative state, as these changes may also represent a reversable response to gradual or rapid changes in external drivers (Ratajczak et al., 2018). Current analyses have confirmed the abrupt changes in the biomass of

the most important stocks of the Adriatic Sea and further investigations will provide important insights regarding the persistence and the underlying causes of these changes, which are crucial components in the detection of state changes (Bestelmeyer et al., 2011).

CONCLUSIONS

In this study, we applied a combination of methods in order to investigate the occurrence of abrupt changes in the biomass of the most important fish stocks in the Adriatic. The combination of different methods allowed us, first to identify the trajectory type that explains better the biological data-series and also detect the location of the past changepoints. Our analysis shows that an abrupt trajectory better explains the dynamics of five out of seven species we investigated, compared to the other trajectories considered. This study marks the first attempt to study the presence of abrupt dynamics in the biomass of the most important commercial stocks in the Adriatic. Further analyses are needed in order to evaluate the persistence of the changes, to identify the drivers, and the ability of management measures to restore the stocks to the previous conditions.

REFERENCES

Bestelmeyer, B.T., Ellison, A.M., Fraser, W.R., Gorman, K.B., Holbrook, S.J., Laney, C.M., Ohman, M.D., Peters, D.P.C., Pillsbury, F.C., Rassweiler, A., Schmitt, R.J., Sharma, S., 2011. Analysis of abrupt transitions in ecological systems. Ecosphere 2, art129. https://doi.org/10.1890/ES11-00216.1

Blöcker, A.M., Gutte, H.M., Bender, R.L., Otto, S.A., Sguotti, C., Möllmann, C., 2023. Regime shift dynamics, tipping points and the success of fisheries management. Sci Rep 13, 1–11. https://doi.org/10.1038/s41598-022-27104-y

Bonacci, O., Vrsalović, A., 2022. Differences in Air and Sea Surface Temperatures in the Northern and Southern Part of the Adriatic Sea. Atmosphere 13, 1158. https://doi.org/10.3390/atmos13071158

Brander, K.M., 2007. Global fish production and climate change. Proc Natl Acad Sci U S A 104, 19709–19714. https://doi.org/10.1073/pnas.0702059104

Britten, G.L., Duarte, C.M., Worm, B., 2021. Recovery of assessed global fish stocks remains uncertain. Proceedings of the National Academy of Sciences 118, e2108532118. https://doi.org/10.1073/pnas.2108532118

Cardinale, M., Osio, G.C., Scarcella, G., 2017. Mediterranean Sea: A Failure of the European Fisheries Management System. Frontiers in Marine Science 4.

Clark, T.J., Luis, A.D., 2020. Nonlinear population dynamics are ubiquitous in animals. Nat Ecol Evol 4, 75–81. https://doi.org/10.1038/s41559-019-1052-6

Coll, M., Piroddi, C., Albouy, C., Ben Rais Lasram, F., Cheung, W.W.L., Christensen, V., Karpouzi, V.S., Guilhaumon, F., Mouillot, D., Paleczny, M., Palomares, M.L., Steenbeek, J., Trujillo, P., Watson, R., Pauly, D., 2012. The Mediterranean Sea under siege: spatial overlap between marine biodiversity, cumulative threats and marine reserves. Global Ecology and Biogeography 21, 465–480. https://doi.org/10.1111/j.1466-8238.2011.00697.x

Colloca, F., Cardinale, M., Maynou, F., Giannoulaki, M., Scarcella, G., Jenko, K., Bellido, J.M., Fiorentino, F., 2013. Rebuilding Mediterranean fisheries: a new paradigm for ecological sustainability. Fish and Fisheries 14, 89–109. https://doi.org/10.1111/j.1467-2979.2011.00453.x

Conversi, A., Dakos, V., Gårdmark, A., Ling, S., Folke, C., Mumby, P.J., Greene, C., Edwards, M., Blenckner, T., Casini, M., Pershing, A., Möllmann, C., 2015. A holistic view of marine regime shifts. Philosophical Transactions of the Royal Society B: Biological Sciences 370, 20130279. https://doi.org/10.1098/rstb.2013.0279

deYoung, B., Barange, M., Beaugrand, G., Harris, R., Perry, R.I., Scheffer, M., Werner, F., 2008. Regime shifts in marine ecosystems: detection, prediction and management. Trends in Ecology & Evolution 23, 402–409. https://doi.org/10.1016/j.tree.2008.03.008

Erdman, C., Emerson, J.W., 2008. bcp: An R Package for Performing a Bayesian Analysis of Change Point Problems. Journal of Statistical Software 23, 1–13. https://doi.org/10.18637/jss.v023.i03

Folke, C., Carpenter, S., Walker, B., Scheffer, M., Elmqvist, T., Gunderson, L., Holling, C.S., 2004. Regime Shifts, Resilience, and Biodiversity in Ecosystem Management. Annu. Rev. Ecol. Evol. Syst. 35, 557–581. https://doi.org/10.1146/annurev.ecolsys.35.021103.105711

Furlan, E., Torresan, S., Critto, A., Lovato, T., Solidoro, C., Lazzari, P., Marcomini, A., 2019. Cumulative Impact Index for the Adriatic Sea: Accounting for interactions among climate and anthropogenic pressures. Science of The Total Environment 670, 379–397. https://doi.org/10.1016/j.scitotenv.2019.03.021

GFCM, 2024. Working Group on Stock Assessment of Small Pelagic Species (WGSASP) session on small pelagic species in the Adriatic Sea (GSAs 17–18) | General Fisheries Commission for the Mediterranean (GFCM) | Food and Agriculture Organization of the United Nations [WWW Document]. URL https://www.fao.org/gfcm/technical-meetings/detail/ar/c/1696873/ (accessed 8.5.24).

Hidalgo, M., Ligas, A., Bellido, J.M., Bitetto, I., Carbonara, P., Carlucci, R., Guijarro, B., Jadaud, A., Lembo, G., Manfredi, C., Esteban, A., Garofalo, G., Ikica, Z., García, C., Sola, L.G. de, Kavadas, S., Maina, I., Sion, L., Vittori, S., Vrgoc, N., 2019a. Size-dependent survival of European hake juveniles in the Mediterranean Sea. Scientia Marina 83, 207–221. https://doi.org/10.3989/scimar.04857.16A

Hidalgo, M., Mihneva, V., Vasconcellos, M., Bernal, M., 2019b. Climate Change Impacts, Vulnerabilities and Adaptations: Mediterranean Sea and the Black Sea Marine Fisheries, in: Impacts of Climate Change on Fisheries and Aquaculture: Synthesis of Current Knowledge, Adaptation and Mitigation Options. Food & Agriculture Org., p. 627.

JRC, 2023. Scientific Technical and Economic Committee for Fisheries (STECF) – Stock Assessments in the Adriatic, Ionian and Aegean Seas and Strait of Sicily (STECF-23-12) [WWW Document]. JRC Publications Repository. https://doi.org/10.2760/194583

Killick, R., Eckley, I.A., 2014. changepoint: An R Package for Changepoint Analysis. Journal of Statistical Software 58, 1–19. https://doi.org/10.18637/jss.v058.i03

Levin, P.S., Möllmann, C., 2015. Marine ecosystem regime shifts: challenges and opportunities for ecosystem-based management. Philosophical Transactions of the Royal Society B: Biological Sciences 370, 20130275. https://doi.org/10.1098/rstb.2013.0275

Lotze, H.K., Coll, M., Dunne, J.A., 2011a. Historical Changes in Marine Resources, Food-web Structure and Ecosystem Functioning in the Adriatic Sea, Mediterranean. Ecosystems 14, 198–222. https://doi.org/10.1007/s10021-010-9404-8

Lotze, H.K., Coll, M., Magera, A.M., Ward-Paige, C., Airoldi, L., 2011b. Recovery of marine animal populations and ecosystems. Trends in Ecology & Evolution 26, 595–605. https://doi.org/10.1016/j.tree.2011.07.008

Marbà, N., Jorda, G., Agusti, S., Girard, C., Duarte, C.M., 2015. Footprints of climate change on Mediterranean Sea biota. Frontiers in Marine Science 2, 56. https://doi.org/10.3389/fmars.2015.00056

Martín, P., Sabatés, A., Lloret, J., Martin-Vide, J., 2012. Climate modulation of fish populations: the role of the Western Mediterranean Oscillation (WeMO) in sardine (Sardina pilchardus) and anchovy (Engraulis encrasicolus) production in the north-western Mediterranean. Climatic Change 110, 925–939. https://doi.org/10.1007/s10584-011-0091-z

Micheli, F., Halpern, B.S., Walbridge, S., Ciriaco, S., Ferretti, F., Fraschetti, S., Lewison, R., Nykjaer, L., Rosenberg, A.A., 2013. Cumulative Human Impacts on Mediterranean and Black Sea Marine Ecosystems: Assessing Current Pressures and Opportunities. PLoS ONE 8, e79889. https://doi.org/10.1371/journal.pone.0079889

Möllmann, C., Cormon, X., Funk, S., Otto, S.A., Schmidt, J.O., Schwermer, H., Sguotti, C., Voss, R., Quaas, M., 2021. Tipping point realized in cod fishery. Sci Rep 11, 14259. https://doi.org/10.1038/s41598-021-93843-z

Möllmann, C., Folke, C., Edwards, M., Conversi, A., 2015. Marine regime shifts around the globe: theory, drivers and impacts. Philos Trans R Soc Lond B Biol Sci 370, 20130260. https://doi.org/10.1098/rstb.2013.0260

O’Leary, J.K., Micheli, F., Airoldi, L., Boch, C., De Leo, G., Elahi, R., Ferretti, F., Graham, N.A.J., Litvin, S.Y., Low, N.H., Lummis, S., Nickols, K.J., Wong, J., 2017. The Resilience of Marine Ecosystems to Climatic Disturbances. BioScience 67, 208–220. https://doi.org/10.1093/biosci/biw161

Pélissié, M., Devictor, V., Dakos, V., 2024. A systematic approach for detecting abrupt shifts in ecological timeseries. Biological Conservation 290, 110429. https://doi.org/10.1016/j.biocon.2023.110429

Piroddi, C., Coll, M., Liquete, C., Macias, D., Greer, K., Buszowski, J., Steenbeek, J., Danovaro, R., Christensen, V., 2017. Historical changes of the Mediterranean Sea ecosystem: modelling the role and impact of primary productivity and fisheries changes over time. Sci Rep 7, 44491. https://doi.org/10.1038/srep44491

Ratajczak, Z., Carpenter, S.R., Ives, A.R., Kucharik, C.J., Ramiadantsoa, T., Stegner, M.A., Williams, J.W., Zhang, J., Turner, M.G., 2018. Abrupt Change in Ecological Systems: Inference and Diagnosis. Trends in Ecology & Evolution 33, 513–526. https://doi.org/10.1016/j.tree.2018.04.013

Santojanni, A., Arneri, E., Bernardini, V., Cingolani, N., Di Marco, M., Russo, A., 2006. Effects of environmental variables on recruitment of anchovy in the Adriatic Sea. Clim. Res. 31, 181–193. https://doi.org/10.3354/cr031181

Scarcella, G., Grati, F., Raicevich, S., Russo, T., Gramolini, R., Scott, R.D., Polidori, P., Domenichetti, F., Bolognini, L., Giovanardi, O., Celić, I., Sabatini, L., Vrgoč, N., Isajlović, I., Marčeta, B., Fabi, G., 2014. Common sole in the northern and central Adriatic Sea: Spatial management scenarios to rebuild the stock. Journal of Sea Research 89, 12–22. https://doi.org/10.1016/j.seares.2014.02.002

Scheffer, M., 2009. Critical Transitions in Nature and Society. Princeton University Press.

Sguotti, C., Bischoff, A., Conversi, A., Mazzoldi, C., Möllmann, C., Barausse, A., 2022. Stable landings mask irreversible community reorganizations in an overexploited Mediterranean ecosystem. Journal of Animal Ecology 91, 2465–2479. https://doi.org/10.1111/1365-2656.13831

Sguotti, C., Cormon, X., 2018. Regime Shifts – A Global Challenge for the Sustainable Use of Our Marine Resources, in: Jungblut, S., Liebich, V., Bode, M. (Eds.), YOUMARES 8 – Oceans Across Boundaries: Learning from Each Other. Springer International Publishing, Cham, pp. 155–166. https://doi.org/10.1007/978-3-319-93284-2_11

Szuwalski, C.S., Vert-Pre, K.A., Punt, A.E., Branch, T.A., Hilborn, R., 2015. Examining common assumptions about recruitment: a meta-analysis of recruitment dynamics for worldwide marine fisheries. Fish and Fisheries 16, 633–648. https://doi.org/10.1111/faf.12083

Turner, M.G., Calder, W.J., Cumming, G.S., Hughes, T.P., Jentsch, A., LaDeau, S.L., Lenton, T.M., Shuman, B.N., Turetsky, M.R., Ratajczak, Z., Williams, J.W., Williams, A.P., Carpenter, S.R., 2020. Climate change, ecosystems and abrupt change: science priorities. Philos Trans R Soc Lond B Biol Sci 375, 20190105. https://doi.org/10.1098/rstb.2019.0105

Tzanatos, E., Raitsos, D.E., Triantafyllou, G., Somarakis, S., Tsonis, A.A., 2014. Indications of a climate effect on Mediterranean fisheries. Climatic Change 122, 41–54. https://doi.org/10.1007/s10584-013-0972-4

Vasilakopoulos, P., Maravelias, C.D., Tserpes, G., 2014. The Alarming Decline of Mediterranean Fish Stocks. Current Biology 24, 1643–1648. https://doi.org/10.1016/j.cub.2014.05.070

EXOPOLYSACCHARIDES PRODUCED FROM LACTIC ACID BACTERIA IN DANGKE CHEESE AND ITS POTENTIAL HEALTH BENEFITS: A REVIEW

R W Kadir[1], R Malaka[2]*

[1]Graduate student of Animal Science, Faculty of Animal Science, Hasanuddin University, Jl. Perintis Kemerdekaan KM. 10, Makassar, 90245, South Sulawesi, Indonesia.
[2]Department of Animal Science, Faculty of Animal Science, Hasanuddin University, Jl. Perintis Kemerdekaan KM. 10, Makassar, 90245, South Sulawesi, Indonesia.

*Corresponding author: malaka_ag39@yahoo.co.id

ABSTRACT

Dangke is a traditional Indonesian cheese originating from Enrekang in South Sulawesi that has gained significant recognition in the dairy sector due to its distinctive qualities and possible advantages for health. Lactic acid bacteria (LAB) are important for creation and maturation of Dangke cheese because they contribute to its unique taste and texture. These LABs generate exopolysaccharide (EPS), a noteworthy influence on the physicochemical and microbiological characteristics of Dangke cheese. LAB-produced EPS in Dangke cheese not only improves the cheese's functional characteristics but also provides significant health benefits. This review provides a comprehensive analysis of the EPS classification and its possible physiological advantages. In addition, EPS has advantages for human health, particularly in relation to the use of functional food. It acts as an antibacterial, antioxidant, cholesterol-lowering, and anticancer agent. The review findings indicate that EPS derived from lactic acid bacteria in Dangke cheese may enhance general well-being and facilitate the creation of functional dairy products with additional health benefits.

Keywords: Dangke, exopolysaccharide, health benefits, lactic acid bacteria.

INTRODUCTION

Dangke is a typical Indonesian cheese that is classified as a variety of fresh, soft cheese originating from Enrekang, South Sulawesi. The production of Dangke cheese involves curdling fresh cow or buffalo milk with papaya enzyme [1, 2]. The addition of papain, which comes from papaya gum, causes a cohesive structure to form in dangke by separating the water and protein. The amount of papain added and the cooking method affect the qualities of Dangke, including its physical, chemical, and organoleptic aspects [3]. Dangke cheese has gained increasing recognition in the dairy sector due to its unique characteristics and potential health benefits. The research conducted by Syah et al. [4] confirmed the presence of lactic acid bacteria in dangke. A study conducted by Mukhlisah et al. [5] successfully identified 30 LAB isolates in dangke. After sequencing the 16S rRNA gene, five of these isolates were identified.

The fermented food sector extensively employs LAB. Lately, there has been an increasing curiosity with the microbial metabolic characteristics of lactic acid bacteria, owing to their significant contribution to the food sector and the advantageous impacts of probiotics [6, 7]. Lactic acid bacteria possess the capacity to decompose complex structures in food, such as indigestible polysaccharides, and transform unpleasant flavor chemicals. In addition, throughout their metabolic processes, they have the ability to generate various compounds such as short-chain fatty acids, bacteriocins, vitamins, amines, and exopolysaccharides [8, 9].

Bacterial exopolysaccharides (EPS) have garnered recent attention for their distinct structure, characteristics, and potential applications in many domains such as science, industry, medicine, and technology [10]. Bacterial exopolysaccharides encompass various categories of polysaccharides. Several of polysaccharides have similarities to plant polysaccharides such cellulose, amylose, amylopectin, and algin. Additional polysaccharides that exhibit resemblances to animal-derived polysaccharides are glycogen and hyaluronic acid [11, 12].

EPS offers numerous practical advantages. Firstly, commercial scale production of EPS is possible. In addition, EPS is harmless and can improve the quality of food, especially when present in little amounts. In addition, EPS does not contribute to environmental contamination because it easily degrades. Moreover, EPS has potential uses in the treatment of several disease and the pharmaceutical sector [13,14]. Scientists in the field of food microbiology are motivated to explore the possible benefits of bacteria in manufacturing EPS due to the rapid progress of human civilization and the limiting supply of arable land. Fermented milk starters can produce exopolysaccharides, which provide advantages over those produced by other microorganisms [11].

The EPS in Dangke cheese comes from lactic acid bacteria and has many benefits, such as being able to stop blood clots, boost the immune system, fight cancer, and introduce new cells into the body. This is believed to be attributed to the production of EPS [15]. The EPS also carries a minor danger of toxin exposure and offers superior quality. EPS generated by LAB functions as a food stabilizer, gelling agent, and immunostimulant, contributing to its role as a nutritious food [16]. This paper will examine the process of producing EPS from LAB derived from Dangke cheese and explore its potential applications in promoting health as a functional food product.

The History of Dangke and Its Manufacturing Process

Indonesia has many kinds of traditional soft cheeses that are typical of the region, and one of them is Dangke, which hails from the Enrekang Regency in South Sulawesi and holds great potential as a traditional culinary additive. Dangke, renowned in Enrekang's culinary scene, is a highly acclaimed product known for its cheese-like flavor and tofu-like soft texture [17, 18].

Figure 1. Dangke [18].

The practice of Dangke processing has been in existence since 1905, transmitted over generations, and continues to endure to this day. The product has expanded its presence in the Enrekang Regency, becoming a prominent food industry at the household level across several places [19]. The word "dangke" originates from the Dutch expression "*dank u well*", which the Dutch employed during the colonial period to convey appreciation when the Enrekang people offered them food [20]. Regrettably, the Enrekang community abbreviated this term to "Dangke" due to its inherent complexity. The word's meaning is "thank you," and it also serves as a designation for a unique variety of soft cheese [21].

Dangke, a classic cheese product, is characterized by its oval shape, dense and chewy texture, powerful milk aroma, and savory taste. Dangke is produced by combining fresh cow or buffalo milk with coagulating enzymes [22]. The traditional method of producing dangke involves heating fresh milk and adding papaya sap as a coagulating enzyme. This results in the formation of two distinct phases, curd (solids) and whey (liquid) [23]. The sap of the papaya tree is extracted by incising the petiole, allowing the sap to flow out. Subsequently, the sap is gathered and stored in a receptacle. The entire quantity of cow's milk is subjected to gentle heating at a temperature of approximately 75–80 °C for a duration of 5 minutes. Subsequently, a solution containing 0.75% (v/v) papaya leaf sap is introduced into the milk while continuously stirring, resulting in the formation of milk tofu. Subsequently, the milk curd is subjected to filtration and compression in order to inhibit the drainage of whey, 1 liter of milk yielded 250 grams of milk curd [24, 25, 26].

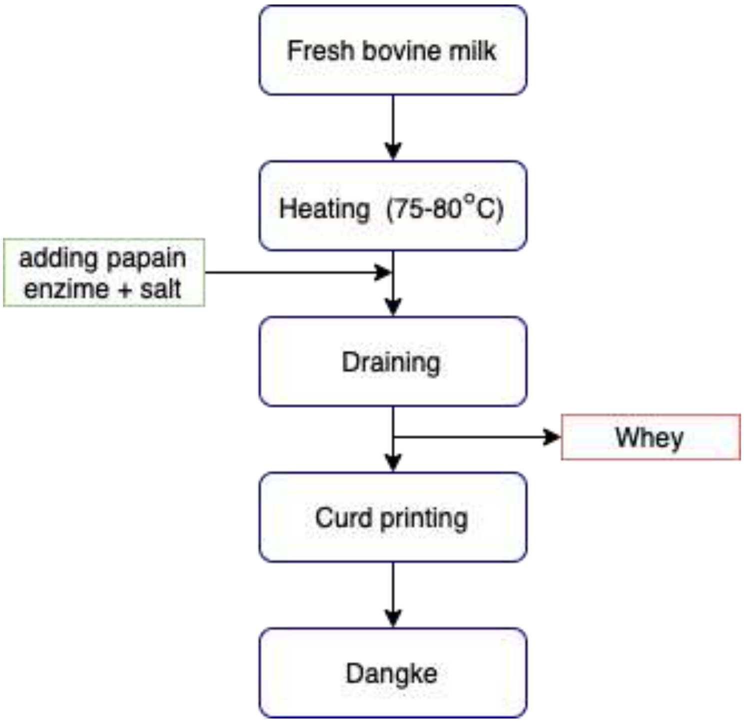


Figure 2. Dangke making processing.

Dangke, a dairy product, has a commendable nutritional composition. Dangke has a macronutrient composition of 14.02% protein, 13.32% fat, 9.72% crude fiber, 4.70% carbs, and 47.94% water [27]. Currently, a wide range of technologies have been implemented to enhance the quality and extend the durability of Dangke products. The utilization of LAB will enhance coagulation and result in dangke with a denser and tender texture. Chemicals that enhance the aroma and flavour of dangke are synthesised during the fermentation process by LAB. LAB is responsible for the synthesis of lactic acid, which results in an increase in the acidity of dangke cheese. The presence of lactic acid in dangke serves as an acidifier, which lowers the pH and inhibits the proliferation of harmful bacteria that might cause spoilage or disease [1, 28, 29]. The incorporation of LAB in the manufacturing process of dangke exhibits significant promise in enhancing product quality, nutritional content, and health advantages. Despite being in the developmental phase, this innovation has the potential to be a significant advancement in modernizing and enhancing the competitiveness of dangke in the food market [30, 31].

Table 1. LABs isolated from Dangke-cheese.

Genus	Strain	Reference
Lactobacillus	*Lactobacillus fermentum*	[4, 32, 33]
	Lactobacillus plantarum	[33, 34]
	Lactobacillus acidhophilus	[34]
	Lactobacillus casei	[35]
	Lactobacillus brevis	[35]
Enterococcus	*Enterococcus faecium*	[36]
Weisella	*Weisella confusa*	[32]

Characterization and Biosynthesis of EPS from LAB

Lactic acid bacteria (LAB) are known for their capacity to produce a wide variety of heteropolysaccharides, commonly known as exopolysaccharides (EPS). These EPS have attracted considerable attention in the food and pharmaceutical industries due to their diverse functional characteristics [37, 38]. The EPS display a diverse array of structural complexity, encompassing differences in monosaccharide content, glycosidic linkages, and branching patterns. Comprehending the process of how these microbial exopolysaccharides are produced, purified, and studied is crucial for their efficient application [39].

The word EPS, established by Sutherland in 1972, encompasses all forms of bacterial polysaccharides found external to the cell wall [40]. EPS production is observed in multiple species of Gram-positive bacteria and Gram-negative LAB, and it has been thoroughly investigated in various study domains. Microorganisms synthesize extracellular polymeric substances (EPS) to create a protective barrier that is impervious to drought, amoebic assault, bacteriophages, and leukocytes [41, 42, 43]. EPS can be categorized into two primary classes according to their content and synthesis mechanism: homopolysaccharides and heteropolysaccharides [31].

EPS are LAB secondary metabolites that are secreted under specific conditions. EPS have the capacity to attach to and colonise the intestinal mucosa, hence inhibiting the growth of harmful bacteria [44]. EPS can be categorised as either heteropolysaccharides or, depending on the structure and composition of monosaccharides [45]. Heteropolysaccharides consist of many monosaccharides that are synthesised intracellularly, whereas homopolysaccharides consist of a singular kind of monosaccharide and are created extracellularly [46]. The main components of EPS are primarily monosaccharides, accompanied by non-carbohydrate molecules such as succinate, phosphate, acetate, and pyruvate [47].

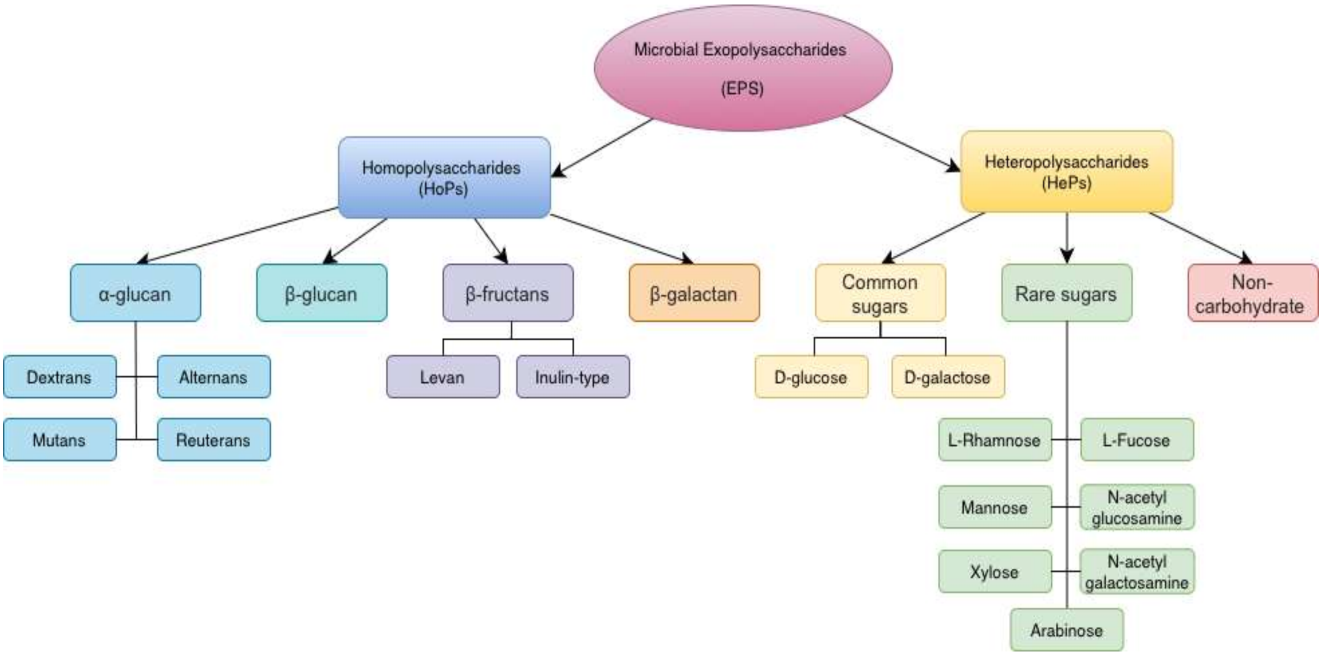


Figure 3. Microbial exopolysaccharides classification.

Homopolysaccharides, such as glucose or fructose, are polymers made up of only one type of monosaccharide. Heteropolysaccharides usually comprise a combination of two to four different monosaccharides. Both categories of polymers can have either a linear or branching structure, and may incorporate acyl or other substituents [48]. Macromolecules that are composed of a singular monosaccharide, such as glucose or fructose, are referred as homopolysaccharides. Additionally, the chains of this particular EPS polymer can be found in either a linear or a branched configuration[11, 49]. Dextran, levans, and curdlan are specific instances of homopolysaccharide EPS. Heteropolysaccharides are polysaccharides composed of a mixture of two to four different monosaccharides, including glucose, galactose, mannose, fucose, and rhamnose [38, 50]. Pullulan is a heteropolysaccharide that belongs to the EPS category. Ongoing research is being conducted on the composition, production, and other elements of early microorganism EPS cultures [40].

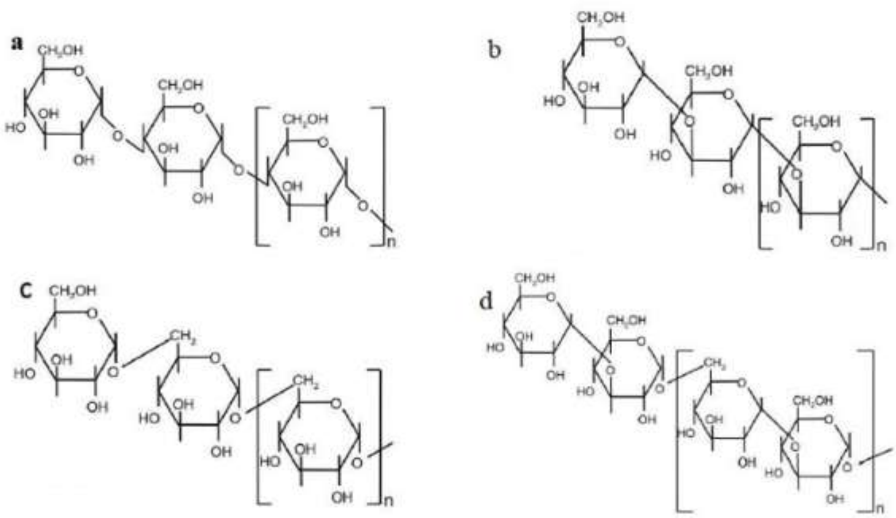


Figure 4. Homopolysaccharides glucan structure a) reutran; b) mutan; c) dextran; and d) alternan [51].

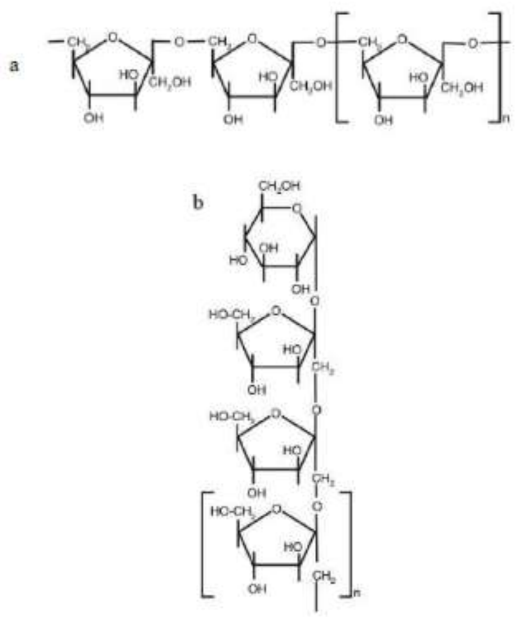


Figure 5. Homopolysaccharides fructan structure a) levan; and b) inulin-type [51].

Some examples of bacteria that can produce homopolysaccharides are *Weissella*, *Leuconostoc*, *Lactobacillus*, and *Pediococcus*. Extracellular enzymes, specifically glucan-sucrase or fructan-sucrase, aid in the synthesis of homopolysaccharides in these bacteria [48]. Both enzymes catalyze the transfer of monosaccharides from specific substrates, resulting in the formation of polysaccharide chains through the hydrolysis of monosaccharides. These monosaccharides are subsequently connected to the glycan acceptor chain. The activity of glucansucrase is regulated by the glycosyltransferase gene (gtf), whereas the activity of fructansucrase is regulated by the fructosyltransferase gene (ftf) [52, 53]. Glucansucrase is a component of the amylase enzyme that functions as a hydrolyzer of glycoside linkages, contributing to the formation of glucan and fructan [46].

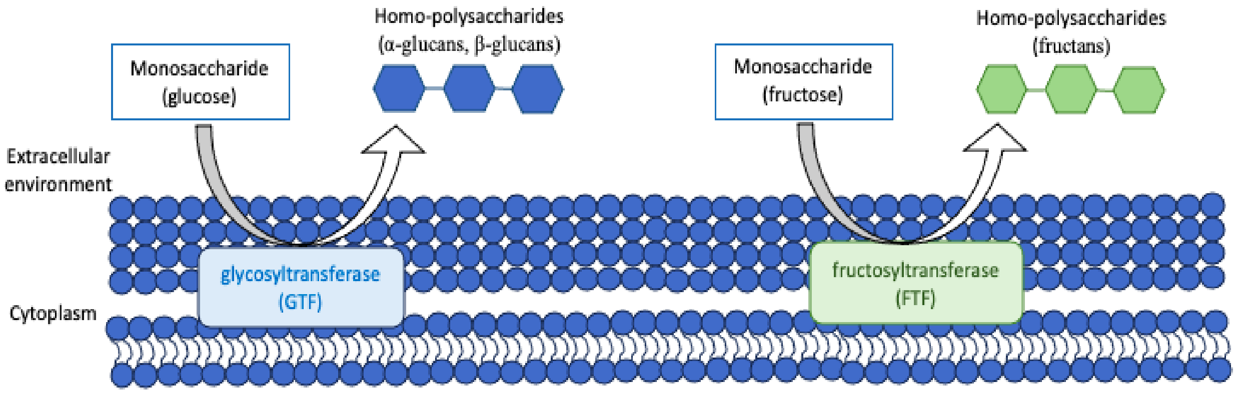


Figure 6. Biosynthesis of homopolysaccharides.

Different types of homopolysaccharides produced by LAB include glucans, fructans, and galactans, which are composed of D-glucose, D-fructose, and D-galactose, respectively [38]. Glucans are created through the arrangement of glucose molecules in a primary structure, featuring different levels of branching and binding sites that differ among different bacterial strains [54]. It is possible to divide glucans into two separate groups, α-glucans and β-glucans. Many different species of *Lactobacillus*, *Leuconostoc*, *Streptococcus*, and *Weissella* produce them [55].

There are four discrete categories of alpha-glucans such as dextrans are water-soluble polysaccharides. Mutans, however, are typically unable to dissolve in water. Reuteran are polysaccharides that are soluble in water. On the other hand, alternans have alternating bonds, resulting in decreased solubility and viscosity in water [38]. Beta-glucan is a type of homopolysaccharide that is synthesized by *Pediococcus* and *Streptococcus spp.* Fructose-based polymers created fructans that are soluble in water. They are made by some strains of bacteria, including *Leuconostoc mesenteroides*, *Streptococcus salivarius*, *Lactobacillus reuteri*, *Lactobacillus johnsonii*, and *Lactobacillus sanfranciscensis* [38, 56, 57]. Water-soluble galactans are not very common and include galactose units bound together in an α-(1→6) configuration. They are created by specific types of LAB, such as *Lactococcus lactis subsp. lactis*, *Lactobacillus delbrueckii subsp. bulgaricus* and *Weissella confusa* [58].

Heteropolysaccharides exhibit a higher level of structural complexity in comparison to homopolysaccharides. This is due to their composition of multiple repeating sugar units, such as N-acetylated monosaccharides (N-acetyl-glucosamine and N-acetyl-galactosamine), hexoses (D-glucose, D-galactose, D-mannose), pentoses (D-ribose, D-arabinose, D-xylose), or uronic acids (D-glucuronic acid, D-galacturonic acid). Heteropolysaccharides can also have branching or be unbranched [51, 59, 60]. Bacteria belonging to the genera *Lactobacillus*, *Lactococcus*, and *Streptococcus* are the ones responsible for the creation of these bacteria. LAB create heteropolysaccharides in limited quantities, although these compounds exhibit significant thickening capabilities even at low concentrations [38, 61].

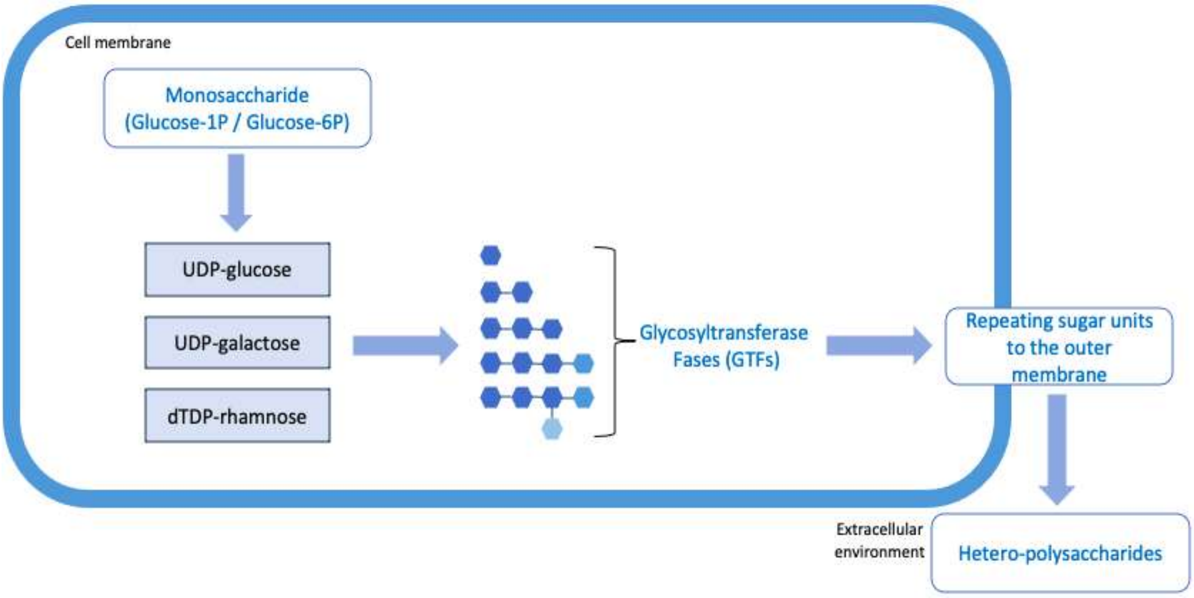


Figure 7. Biosynthesis of heteropolysaccharides.

Heteropolysaccharide synthesis entails a series of several steps: 1. Sugars are carried into the cytoplasm of bacterial cells and undergo phosphorylation to become either glucose-1-phosphate or glucose-6-phosphate; 2. Within the cell, uridine-diphosphate-glucose (UDP-glucose), UDP-galactose, and deoxythymidine-diphospho-mammnose (dTDP-rhamnose) nucleotides are produced; 3. The cell membrane contains undecaprenol diphosphate (UDA) anchors to which individual repeat units are linked. These repeat units are generated using numerous GTFs; 4. The repeat sugar units are then transported to the outer membrane; 5. In the end, the sugar molecules are joined together to create intricate carbohydrates called heteropolysaccharides. These are subsequently released into the extracellular environment as EPS. EPS molecules attach to the cell wall either as a capsule or as a sticky polymer. The genes responsible for the production of heteropolysaccharides in thermophilic Lactobacillus are acquired by chromosomal replication, but in mesophilic *Lactobacillus*, these genes are gained from plasmid replication. The same genes have a regulatory function in the production of heteropolysaccharides [53, 62, 63].

Potential Health Benefits of EPS

Lactic acid bacteria (LAB) are a heterogeneous group of microorganisms that have gained recognition for their versatility and importance in various industries, including food production, biotechnology, and medicine [64, 65]. These bacteria have the ability to produce exopolysaccharides (EPS), complex carbohydrate polymers that have attracted significant attention due to their potential physiological benefits [66, 67].

The therapeutic uses of EPS generated by LAB include anticancer activity, immune-boosting action, and the ability to lower blood cholesterol levels [38, 68]. These biologically active compounds have also been studied as possible biothickeners in the food and dairy sectors, offering powerful emulsifying capabilities that are crucial in numerous food formulations [64, 68].

Gaining insight into the mechanisms that drive the biosynthesis of these exopolysaccharides is crucial for enhancing production and fully exploring their capabilities [69]. Scientists have examined the intricate genetic and metabolic processes involved in the production of these external polysaccharides. This research has opened up the possibility of making genetic changes that could lead to higher production levels and enhanced performance [65].

EPS derived from LAB exhibit exceptional versatility and feature health-enhancing properties, rendering them highly sought-after natural constituents in the culinary, pharmaceutical, and medical sectors [70]. The on-site generation of EPS by food-grade cultures is a promising substitute for conventional additives, as it fulfils the increasing consumer need for natural and clean-label products. This approach offers similar rheological and sensory properties while also providing additional health benefits [64, 71].

Antimicrobial agents

EPS synthesized by LAB have demonstrated antibacterial properties against a range of diseases. These characteristics provide them appealing options for natural preservatives and prospective substitutes for synthetic antimicrobials [72]. EPS has the ability to disrupt the process of biofilm development in harmful bacteria. They have the ability to vie for attachment sites or disrupt intercellular communication (quorum sensing) that is necessary for the production of biofilms [73].

Certain EPS demonstrate direct bacteriostatic or bactericidal properties against microorganisms. This could be attributed to the chemical composition or distinct functional groups present in the EPS. EPS can augment the efficacy of other antimicrobial compounds generated by LAB, such as bacteriocins or organic acids [37, 73, 74]. EPS has the ability to activate the immune system of the host, so indirectly aiding in the management of pathogens. This encompasses the stimulation of macrophages and heightened synthesis of antimicrobial peptides[68, 75]

EPS has the ability to create a protective barrier on surfaces, which hinders the attachment and growth of harmful microorganisms. Certain EPS (extracellular polymeric substances) can cause acidity of the surrounding environment, which creates adverse conditions for numerous infections. EPS has the ability to attach to vital nutrients, rendering them inaccessible to harmful microorganisms and impeding their proliferation. Specific EPS have demonstrated the ability to damage the cell membrane of pathogens, resulting in either cell death or growth inhibition. Certain EPS demonstrate antiviral capabilities, which can potentially impede the attachment or penetration of viruses into host cells [76].

EPS derived from certain LAB strains have demonstrated potent antifungal properties, particularly against fungi that cause food deterioration. The antibacterial efficacy of extracellular polymeric substances (EPS) can exhibit substantial variation among various strains of LAB. Notable LAB strains that produce EPS with significant antibacterial properties include *Lactobacillus plantarum, Lactobacillus rhamnosus, Streptococcus thermophilus, Lactobacillus bulgaricus*, and *Weissella confusa*. The EPS from LAB has demonstrated antimicrobial efficacy against a range of pathogens, such as *Escherichia coli, Salmonella spp, Listeria monocytogenes, Staphylococcus aureus*, and *Candida albicans* [71, 77, 78]. The in vitro test shown that the EPS produced by *L. plantarum* C70, which was isolated from camel milk, resulted in a significant reduction of 2-3 logs in the viability of *E.coli* and *S. aureus* when exposed to these bacterial pathogens [79]. The EPS derived from *L. rhamnosus,* which was obtained from human breast milk, exhibits potent antibacterial effects against *E. coli* (12–14.3 mm) and *S. typhimurium* (10–13 mm). Additionally, it demonstrates remarkable prevention of S. typhimurium biofilm formation (58–71%) under laboratory conditions [80].

Possible uses of the antimicrobial properties of EPS from LAB include natural food preservatives, food preservatives, and bioactive packaging materials. Topical antimicrobial agents are substances used in cosmetics or medical applications to kill or inhibit the growth of microorganisms on the skin's surface [81]. Possible substitute or addition to traditional antibiotics The antibacterial efficacy of EPS can be modified by several factors, including the particular structure and chemical composition of EPS, ambient circumstances such as pH and temperature, the microorganisms being targeted, and the concentration of EPS [40]. While numerous research have demonstrated the antibacterial capabilities of EPS derived from LAB, the majority of these investigations were conducted in controlled laboratory environments or within food systems. Additional investigation is required to comprehensively comprehend their efficacy within a living organism and their prospective utilization in therapeutic environments.

Antioxidant

EPS derived from LAB exhibits antioxidant properties and holds potential as a significant attribute with health-related applications [82]. EPS has the ability to directly counteract different types of free radicals, including superoxide anion, hydroxyl radical, and DPPH (2,2-diphenyl-1-picrylhydrazyl) [83]. This capability aids in safeguarding cells against oxidative harm induced by reactive oxygen species. Several EPS have the ability to attach to metal ions, such as iron and copper. EPS inhibits the involvement of these metals in free radical-generating processes, such as the Fenton reaction, by chelating them [84, 85, 86].

EPS frequently demonstrate the ability to decrease the oxidation state of other compounds, enabling them to transfer electrons to reactive substances. The measurement of this feature is typically conducted using assays such as the iron-reducing antioxidant power test (FRAP). EPS has the ability to safeguard crucial cellular constituents, such as lipids, proteins, and DNA, against oxidative harm. This protective effect aids in preserving the structural and functional integrity of cells [87, 88]. Several studies indicate that EPS may enhance the synthesis or function of the body's antioxidant enzymes. The enzymes that are included in this group are superoxide dismutase (SOD), catalase, and glutathione peroxidase. The chemical structure of EPS is frequently credited for its antioxidant action [89]. Antioxidant capacity can be influenced by factors such as molecular weight, monosaccharide content, and the presence of functional groups, such as hydroxyl groups. EPS has potential to enhance the effects of other antioxidants in both cellular and dietary settings, and this could enhance the overall antioxidant capacity [76, 90].

Several EPS derived from LAB maintain their antioxidant properties under different temperature and pH conditions. Their stability renders them potentially valuable in different applications, such as food processing [91]. The antioxidant capacity of EPS may vary considerably across various LAB strains, even within the same species. Some LAB strains that are known for producing EPS with significant antioxidant activity include *Lactobacillus plantarum, Lactobacillus helveticus, Streptococcus thermophilus,* and *Lactobacillus casei*. *Weissella cibaria* is a species of bacteria identified [83, 89]. The EPS produced by L. *fermentum, L. plantarum*, and *L. paracasei*, which were isolated from commercial yogurt, had significant antioxidant properties in terms of their ability to scavenge DPPH free radicals, reduce ferric ions, and contain high levels of total phenolic compounds [92].

Possible uses of the antioxidant properties of EPS derived from LAB encompass Food preservation: utilizing natural antioxidants to prolong the duration of freshness. Functional foods and nutraceuticals aim to offer advantageous effects on health. Cosmetics are used in goods that aim to combat the effects of aging and protect the skin. Possible medical applications include the management of disorders related to oxidative stress [93, 94, 95]. It is crucial to acknowledge that while numerous in vitro studies have shown the antioxidant capabilities of EPS from LAB, additional study is required to comprehensively comprehend its efficacy in vivo and its potential uses in human health. The antioxidant properties of EPS may differ depending on the structure, concentration, and oxidative stress model employed in the study.

Immunomodulatory

EPS are complex carbohydrates with a large molecular size that are released by microbes into their external surroundings. Their immunomodulatory activities have garnered considerable attention, as they can impact the immune system in diverse manners [96]. The immunological response is a vital component of the body's defense mechanism against foreign particles, encompassing both acquired immunity and innate immunity [97]. The immune system is composed of various components, such as humoral immunity which involves antibodies, physical barriers, and cellular immunity which involves leukocytes that can communicate with each other using cytokines [98].

Recently, there has been a surge in scientific investigation into the biological properties of microbial exopolysaccharides, emphasizing their potential for use in the field of biomedicine [39, 90, 99]. Polysaccharides, obtained from microbial sources, have demonstrated the ability to regulate the immune system by stimulating or enhancing the activity of T-cells and macrophages, hence raising the activity of interleukins. This has the potential to result in elevated quantities of antibodies and improved modulation of immune function [100].

EPS derived from LAB has the ability to activate macrophages, dendritic cells, and natural killer cells. This stimulation results in an augmentation of phagocytosis and the generation of cytokines and chemokines. EPS can disrupt the equilibrium between proinflammatory and anti-inflammatory cytokines, leading to an upregulation of anti-inflammatory cytokines such IL-10 and TGF-β [100, 101, 102]. EPS can function as adjuvants, hence enhancing the efficacy of vaccines. They have the ability to enhance the growth and reproduction of B-cells and the generation of antibodies.

Certain EPS demonstrate antioxidant properties, which help to decrease oxidative stress on immune cells. It has the potential to enhance the functionality and lifespan of immune cells. EPS has the ability to attach to Toll-like receptors (TLRs) and other receptors that recognize specific patterns. These contacts can initiate signaling cascades that regulate immune responses [39, 89]. Furthermore, EPS has the ability to regulate adaptive and acquired immune responses, safeguard against gastrointestinal disorders, and modify the activity of the immune system by regulating cytokines [103].

The immunomodulatory effects of EPS can vary based on their origin, composition, and molecular weight. It should be emphasized that various EPS can have varying or even contradictory impacts on the immune system. The immunomodulatory qualities of EPS make it a highly intriguing option for a range of applications, such as the treatment of inflammatory and autoimmune illnesses, as well as the use of prebiotics in functional foods.

Antitumor activity

EPS have the ability to directly trigger apoptosis or programmed cell death in cancer cells. They have the ability to disturb the structure of cell membranes or hinder the metabolic processes within tumor cells [104]. EPS have the ability to augment the function of natural killer (NK) cells and cytotoxic T-lymphocytes, which play a crucial role in monitoring and eradicating tumors. They have the ability to enhance the production of cytokines that decrease tumor growth, such as IFN-γ and TNF-α ([96, 104]. Specific exopolysaccharides have the ability to hinder the development of fresh blood vessels (angiogenesis) that are necessary for the growth and spread of malignancies. One way to accomplish this is by inhibiting pro-angiogenic factors like VEGF [105].

EPS possess the capacity to enhance the effectiveness of traditional chemotherapy medications while minimizing their adverse effects. They have the potential to combat medication resistance in certain types of malignancies. EPS has the potential to disrupt important signaling pathways that play a role in the advancement of cancer, such as NF-κB, MAPK, and PI3K/AKT pathways [106, 107].

Certain EPS (extracellular polysaccharides) have the ability to stimulate macrophages, enhancing their ability to destroy tumors and improving their ability to display tumor antigens. Specific EPS have demonstrated the ability to trigger cell cycle arrest in cancer cells, hence inhibiting their proliferation. EPS with demonstrated anticancer properties encompass EPS derived from lactic acid bacteria, specifically species like *Lactobacillus* and *Bifidobacterium* [38, 108]. Several studies indicate that EPS has the capacity to impact epigenetic pathways, which could potentially reverse abnormal epigenetic alterations in cancer cells. The anticancer activity of EPS can significantly differ based on their origin, composition, and molecular weight.

CONCLUSION

The identification of lactic acid bacteria (LAB) in Dangke, as determined by many investigations, is essential for its production and characteristics. The LAB have the ability to generate exopolysaccharides (EPS), which have attracted interest due to their wide range of uses in the fields of research, industry, medicine, and technology.

The EPS generated by LAB in Dangke cheese provides multiple benefits. At low concentrations, it has the ability to improve the quality of food, and its biodegradability makes it environmentally friendly. Additionally, it shows promise in disease treatment and has prospective uses in the pharmaceutical industry. The EPS derived from Dangke cheese offers several health benefits, such as antibacterial, antioxidant, cholesterol-lowering, and anticancer properties.

In addition, the synthesis of EPS by LAB in fermented milk starters, such those found in Dangke, offers benefits compared to EPS produced by other microbes. Dangke shows great potential as a candidate for the production of functional food. EPS can be utilized as a food stabilizer, gelling agent, and immunostimulant. The increasing fascination in the microbial metabolic characteristics of LAB, together with the potential of EPS in Dangke cheese, creates new opportunities for study and advancement in the food and health sectors. Further research aimed at maximizing EPS synthesis from LAB produced from Dangke cheese and investigating its potential uses in functional food products has the potential to drive groundbreaking advancements in nutrition and health promotion.

ACKNOWLEDGMENT

The author would like to thank for PMDSU scholarship support by Ministry of Education, Culture, Research and Technology. Author would like to express gratitude also to Directorate of Research, Technology and Community Service (DRTPM) Ministry of Education, Culture, Research and Technology for funded this study under contract number: 02035/UN4.22.2/PT.01.03/2024.

REFERENCE

[1] R. Malaka *et al.*, "Evaluation Dangke Cheese Processing by Edible Film Coating Made from Whey Combined with Konjac Flour," *J Food Process Preserv*, vol. 2024, pp. 1–11, Mar. 2024, doi: 10.1155/2024/4676923.

[2] A. N. Al-Baarri, A. M. Legowo, S. K. Arum, and S. Hayakawa, "Extending Shelf Life of Indonesian Soft Milk Cheese (Dangke) by Lactoperoxidase System and Lysozyme," *Int J Food Sci*, vol. 2018, pp. 1–7, May 2018, doi: 10.1155/2018/4305395.

[3] F. M. V. Kesuma, S. M. Sayuthi, A. N. Al-Baari, and A. M. Legowo, "Karakteristik dangke dari susu dengan waktu inkubasi berbeda pasca perendaman dalam larutan laktoferin.," *Jurnal Aplikasi Teknologi Pangan*, vol. 2, no. 3, pp. 155–158, 2013.

[4] S. P. Syah, C. Sumantri, I. I. Arief, and E. Taufik, “Isolation and Identification of Indigenous Lactic Acid Bacteria by Sequencing the 16S rRNA from Dangke, A Traditional Cheese from Enrekang, South Sulawesi,” *Pakistan Journal of Nutrition*, vol. 16, no. 5, pp. 384–392, Apr. 2017, doi: 10.3923/pjn.2017.384.392.

[5] A. N. Mukhlisah, I. I. Arief, and E. Taufik, “Physical, Microbial, and Chemical Qualities of Dangke Produced by Different Temperatures and Papain Concentrations,” *Media Peternakan Fakultas Peternakan Institut Pertanian Bogor*, vol. 40, no. 1, pp. 63–70, Apr. 2017, doi: 10.5398/medpet.2017.40.1.63.

[6] A. García-Ruiz, D. González de Llano, A. Esteban-Fernández, T. Requena, B. Bartolomé, and M. V. Moreno-Arribas, “Assessment of probiotic properties in lactic acid bacteria isolated from wine,” *Food Microbiol*, vol. 44, pp. 220–225, Dec. 2014, doi: 10.1016/j.fm.2014.06.015.

[7] B. N. Abdul Hakim, N. J. Xuan, and S. N. H. Oslan, “A Comprehensive Review of Bioactive Compounds from Lactic Acid Bacteria: Potential Functions as Functional Food in Dietetics and the Food Industry,” *Foods*, vol. 12, no. 15, p. 2850, Jul. 2023, doi: 10.3390/foods12152850.

[8] Z. Liang, K. Zheng, G. Xie, X. Luo, and H. Li, “Sugar Utilization-Associated Food-Grade Selection Markers in Lactic Acid Bacteria and Yeast,” *Pol J Microbiol*, vol. 73, no. 1, pp. 3–10, Mar. 2024, doi: 10.33073/pjm-2024-011.

[9] L. Wang *et al.*, “Analysis of physicochemical properties of exopolysaccharide from Leuconostoc mesenteroides strain XR1 and its application in fermented milk,” *LWT*, vol. 146, p. 111449, Jul. 2021, doi: 10.1016/j.lwt.2021.111449.

[10] A. I. Netrusov, E. V. Liyaskina, I. V. Kurgaeva, A. U. Liyaskina, G. Yang, and V. V. Revin, “Exopolysaccharides Producing Bacteria: A Review,” *Microorganisms*, vol. 11, no. 6, p. 1541, Jun. 2023, doi: 10.3390/microorganisms11061541.

[11] R. Malaka, “Bacterial exopolysaccharides production and their roles for human life,” *IOP Conf Ser Earth Environ Sci*, vol. 788, no. 1, p. 012109, Jun. 2021, doi: 10.1088/1755-1315/788/1/012109.

[12] K. Paschalidis *et al.*, “A Polysaccharide-Based Integrated Nutrient Management System Enhances the Antioxidant Properties in Origanum dictamnus (Lamiaceae), a Valuable Local Endemic Plant of Crete,” *Polysaccharides*, vol. 5, no. 1, pp. 28–48, Feb. 2024, doi: 10.3390/polysaccharides5010003.

[13] N. Kaur and P. Dey, “Bacterial exopolysaccharides as emerging bioactive macromolecules: from fundamentals to applications,” *Res Microbiol*, vol. 174, no. 4, p. 104024, May 2023, doi: 10.1016/j.resmic.2022.104024.

[14] M. C. S. Barcelos, K. A. C. Vespermann, F. M. Pelissari, and G. Molina, “Current status of biotechnological production and applications of microbial exopolysaccharides,” *Crit Rev Food Sci Nutr*, vol. 60, no. 9, pp. 1475–1495, May 2020, doi: 10.1080/10408398.2019.1575791.

[15] R. Malaka, E. Abustam, and S. Baco, “Antitumor Activity (In-vitro) of Extracellular Polysaccharide Produced by Ropy Lactobacillus delbrueckii ssp. bulgaricus Isolated from Tradisional Fermented Milk,” *International Journal of Chemistry and Pharmaceutical Sciences*, vol. 4, no. 5, pp. 246–249, May 2016.

[16] R. Malaka and A. Laga, “Isolasi dan Identifikasi Lactobacillus bulgaricus Strain Ropy dari Yoghurt Komersial,” *Sains dan Teknologi*, vol. 5, no. 1, pp. 50–58, Apr. 2005.

[17] S. Rahman, Lasumange, and A. Yunus, “Historical overview and contribution of Dangke as traditional food to income and public health,” *Food Res*, vol. 7, no. 5, pp. 245–250, Oct. 2023, doi: 10.26656/fr.2017.7(5).964.

[18] M. Sa’pang *et al.*, “An Explorative Study of Indonesian Traditional Cheese ‘Dangke’ as High Protein Food from Enrekang, South Sulawesi,” *Poltekita : Jurnal Ilmu Kesehatan*, vol. 17, no. 3, pp. 955–965, Nov. 2023, doi: 10.33860/jik.v17i3.3345.

[19] W. Hatta, M. B. Sudarwanto, I. Sudirman, and R. Malaka, “Survey on the Potency of Cow Milk Dangke as an Alternative Buffalo Milk Dangke in Enrekang, South Sulawesi,” *JITP*, vol. 3, no. 1, pp. 40–50, Jul. 2013.

[20] M. Yusuf, U. Nur Fitriani, Syahriati, R. Saleh, Tasir, and Syamsuar, “Dangke: Local Indigenous Cheese from Enrekang, South Sulawesi Indonesia,” *IOP Conf Ser Earth Environ Sci*, vol. 1097, no. 1, p. 012064, Oct. 2022, doi: 10.1088/1755-1315/1097/1/012064.

[21] R. Malaka, S. Syabil, and F. Maruddin, “Physico-chemical properties and microstructure of Dangke cheese by inoculated of Lactococcus lactis at different temperature storage.,” *The Business and Management Review*, vol. 9, no. 2, pp. 292–298, 2017.

[22] N. Fathiaturrahma, Kusdianawati, and L. Suharli, “Dangke Hasil Olahan dari Susu Kuda Liar Sumbawa, Susu Sapi Sumbawa, dan Susu Kerbau Sumbawa,” *Anoa: Journal of Animal Husbandry*, vol. 1, no. 2, pp. 88–97, Aug. 2022, doi: 10.24252/anoa.v1i2.31054.

[23] N. I. Musra, S. Yasni, and E. Syamsir, “KARAKTERISASI KEJU DANGKE MENGGUNAKAN ENZIM PAPAIN KOMERSIAL DAN PERUBAHAN FISIK SELAMA PENYIMPANAN,” *Jurnal Teknologi dan Industri Pangan*, vol. 32, no. 1, pp. 27–35, Jun. 2021, doi: 10.6066/jtip.2021.32.1.27.

[24] R. Malaka, W. Hatta, and S. Baco, “Evaluation of using edible coating and ripening on Dangke, a traditional cheese of Indonesia,” *Food Res*, vol. 1, no. 2, pp. 51–56, Mar. 2017, doi: 10.26656/fr.2017.2.006.

[25] S. Sulmiyati and N. S. Said, “Karakteristik Dangke Susu Kerbau dengan Penambahan Crude Papain Kering,” *agriTECH*, vol. 38, no. 3, p. 345, Mar. 2019, doi: 10.22146/agritech.24331.

[26] F. Noviatanti Nabilah, S. Listiyowati, and R. I. Astuti, “Diversitas Pangan Fermentasi Berbasis-Susu di Indonesia dan Kandungan Gizinya,” *Jurnal Ilmu Pertanian Indonesia*, vol. 27, no. 4, pp. 552–561, Oct. 2022, doi: 10.18343/jipi.27.4.552.

[27] S. Umarella, A. R. Bamahry, N. F. A. Polanunu, I. D. Kartika, S. Safitri, and S. D. Pramono, “Analisis Kandungan Makronutrien Makanan Tradisional Dangke,” *Fakumi Medical Journal*, vol. 3, no. 11, pp. 829–835, 2023.

[28] Ç. B. Albayrak and M. Duran, “Isolation and characterization of aroma producing lactic acid bacteria from artisanal white cheese for multifunctional properties,” *LWT*, vol. 150, p. 112053, Oct. 2021, doi: 10.1016/j.lwt.2021.112053.

[29] J. Mandha *et al.*, “Effect of Lactic Acid Fermentation on Volatile Compounds and Sensory Characteristics of Mango (Mangifera indica) Juices,” *Foods*, vol. 11, no. 3, p. 383, Jan. 2022, doi: 10.3390/foods11030383.

[30] T. Bintsis, “Lactic acid bacteria as starter cultures: An update in their metabolism and genetics,” *AIMS Microbiol*, vol. 4, no. 4, pp. 665–684, 2018, doi: 10.3934/microbiol.2018.4.665.

[31] R. Malaka, F. Maruddin, and S. Baco, *Dangke Keju Tradisional Enrekang, Sulawesi Selatan*. Makassar: Unhas Press, 2022.

[32] N. J. N. Djide, R. M. Asri, and N. Djide, “Sourcing new potential bacteriocin-producing bacteria from dangke, ethnic cheese of Enrekang, Indonesia,” *IOP Conf Ser Earth Environ Sci*, vol. 575, no. 1, p. 012035, Oct. 2020, doi: 10.1088/1755-1315/575/1/012035.

[33] F. Nur, H. Hafsan, and A. Wahdiniar, “Isolasi Bakteri Asam Laktat Berpotensi Probiotik Pada Dangke, Makanan Tradisional dari Susu Kerbau di Curio Kabupaten Enrekang,” *Biogenesis: Jurnal Ilmiah Biologi*, vol. 3, no. 1, pp. 60–65, Jun. 2015, doi: 10.24252/bio.v3i1.568.

[34] N. Kaswi, M. Hatta, and R. Sjahril, “The Use of Multiplex PCR Reactions to Identification of Lactic Acid Bacteria of Dangke,” *Berkala Kedokteran*, vol. 19, no. 1, p. 79, Feb. 2023, doi: 10.20527/jbk.v19i1.15723.

[35] M. A. Zakariah, R. Malaka, A. Laga, and A. Ako, “Isolation and Identification of Lactic Acid Bacteria from Dangke a White Soft Traditional Cheese from Enrekang Regency,” *International Journal of Recent Technology and Engineering (IJRTE)*, vol. 8, no. 2, pp. 4148–4151, Jul. 2019, doi: 10.35940/ijrte.B3160.078219.

[36] A. Razak, A. Patong, T. Harlim, M. Djide, H. Hasliah, and M. Mahdalia, “Produksi Senyawa Bakteriosin Secara Fermentasi Menggunakan Isolat BAL Enterococcus faecium DU55 dari Dangke,” *Jurnal Akta Kimia Indonesia (Indonesia Chimica Acta)*, vol. 2, no. 2, pp. 1–10, 2016.

[37] T. Harutoshi, “Exopolysaccharides of Lactic Acid Bacteria for Food and Colon Health Applications,” in *Lactic Acid Bacteria - R & D for Food, Health and Livestock Purposes*, InTech, 2013. doi: 10.5772/50839.

[38] D. Jurášková, S. C. Ribeiro, and C. C. G. Silva, “Exopolysaccharides Produced by Lactic Acid Bacteria: From Biosynthesis to Health-Promoting Properties,” *Foods*, vol. 11, no. 2, p. 156, Jan. 2022, doi: 10.3390/foods11020156.

[39] F. Salimi and P. Farrokh, “Recent advances in the biological activities of microbial exopolysaccharides,” *World J Microbiol Biotechnol*, vol. 39, no. 8, p. 213, Aug. 2023, doi: 10.1007/s11274-023-03660-x.

[40] O. N. Tiwari, S. Sasmal, A. K. Kataria, and I. Devi, “Application of microbial extracellular carbohydrate polymeric substances in food and allied industries,” *3 Biotech*, vol. 10, no. 5, p. 221, May 2020, doi: 10.1007/s13205-020-02200-w.

[41] E. Zubaidah, Y. M. Suryawira, and E. Saparianti, “Comparative Study Production of Exopolysaccharide (EPS) by Lactic Acid Bacteria (L. casei and L. plantarum) in Different Media (Dates and Mulberry juice),” *Agroindustrial Journal Vol. 3 Issue 1 (2014) 107-111*, vol. 3, no. 1, pp. 107–111, 2014.

[42] R. Malaka, F. Maruddin, Z. Dwyana, and M. V Vargas, “Assessment of exopolysaccharide production by Lactobacillus delbrueckii subsp. bulgaricus ropy strain in different substrate media.,” *Food Sci Nutr*, vol. 8, no. 3, pp. 1657–1664, Jan. 2020, doi: 10.1002/fsn3.1452.

[43] P.-T. Nguyen, T.-T. Nguyen, D.-C. Bui, P.-T. Hong, Q.-K. Hoang, and H.-T. Nguyen, “Exopolysaccharide production by lactic acid bacteria: the manipulation of environmental stresses for industrial applications,” *AIMS Microbiol*, vol. 6, no. 4, pp. 451–469, 2020, doi: 10.3934/microbiol.2020027.

[44] D. A. Patten and A. P. Laws, “Lactobacillus-produced exopolysaccharides and their potential health benefits: a review,” *Benef Microbes*, vol. 6, no. 4, pp. 457–472, Aug. 2015, doi: 10.3920/BM2014.0117.

[45] S. Patel, A. Majumder, and A. Goyal, “Potentials of Exopolysaccharides from Lactic Acid Bacteria,” *Indian J Microbiol*, vol. 52, no. 1, pp. 3–12, Mar. 2012, doi: 10.1007/s12088-011-0148-8.

[46] M. Guérin, C. R.-D. Silva, C. Garcia, and F. Remize, “Lactic Acid Bacterial Production of Exopolysaccharides from Fruit and Vegetables and Associated Benefits,” *Fermentation*, vol. 6, no. 4, p. 115, Nov. 2020, doi: 10.3390/fermentation6040115.

[47] K. Nouha, R. S. Kumar, S. Balasubramanian, and R. D. Tyagi, “Critical review of EPS production, synthesis and composition for sludge flocculation,” *Journal of Environmental Sciences*, vol. 66, pp. 225–245, Apr. 2018, doi: 10.1016/j.jes.2017.05.020.

[48] M. Nabot, M. Guérin, D. Sivakumar, F. Remize, and C. Garcia, “Variability of Bacterial Homopolysaccharide Production and Properties during Food Processing,” *Biology (Basel)*, vol. 11, no. 2, p. 171, Jan. 2022, doi: 10.3390/biology11020171.

[49] M. Tieking and M. G. Gänzle, “Exopolysaccharides from cereal-associated lactobacilli,” *Trends Food Sci Technol*, vol. 16, no. 1–3, pp. 79–84, Jan. 2005, doi: 10.1016/j.tifs.2004.02.015.

[50] R. Khan, M. D. Shah, L. Shah, P.-C. Lee, and I. Khan, “Bacterial polysaccharides—A big source for prebiotics and therapeutics,” *Front Nutr*, vol. 9, Nov. 2022, doi: 10.3389/fnut.2022.1031935.

[51] G. M. Daba, M. O. Elnahas, and W. A. Elkhateeb, “Contributions of exopolysaccharides from lactic acid bacteria as biotechnological tools in food, pharmaceutical, and medical applications,” *Int J Biol Macromol*, vol. 173, pp. 79–89, Mar. 2021, doi: 10.1016/j.ijbiomac.2021.01.110.

[52] J. Schmid, V. Sieber, and B. Rehm, “Bacterial exopolysaccharides: biosynthesis pathways and engineering strategies,” *Front Microbiol*, vol. 6, May 2015, doi: 10.3389/fmicb.2015.00496.

[53] H. M. Sørensen *et al.*, “Exopolysaccharides of Lactic Acid Bacteria: Production, Purification and Health Benefits towards Functional Food,” *Nutrients*, vol. 14, no. 14, p. 2938, Jul. 2022, doi: 10.3390/nu14142938.

[54] N. Manabe and Y. Yamaguchi, “3D Structural Insights into β-Glucans and Their Binding Proteins,” *Int J Mol Sci*, vol. 22, no. 4, p. 1578, Feb. 2021, doi: 10.3390/ijms22041578.

[55] L. Yu *et al.*, “Purification, characterization and probiotic proliferation effect of exopolysaccharides produced by Lactiplantibacillus plantarum HDC-01 isolated from sauerkraut,” *Front Microbiol*, vol. 14, Jun. 2023, doi: 10.3389/fmicb.2023.1210302.

[56] M. L. Werning *et al.*, “Biological Functions of Exopolysaccharides from Lactic Acid Bacteria and Their Potential Benefits for Humans and Farmed Animals,” *Foods*, vol. 11, no. 9, p. 1284, Apr. 2022, doi: 10.3390/foods11091284.

[57] C. M. Nicolescu *et al.*, “Biopolymers Produced by Lactic Acid Bacteria: Characterization and Food Application,” *Polymers (Basel)*, vol. 15, no. 6, p. 1539, Mar. 2023, doi: 10.3390/polym15061539.

[58] D. Kavitake, P. B. Devi, S. P. Singh, and P. H. Shetty, “Characterization of a novel galactan produced by Weissella confusa KR780676 from an acidic fermented food,” *Int J Biol Macromol*, vol. 86, pp. 681–689, May 2016, doi: 10.1016/j.ijbiomac.2016.01.099.

[59] F. Mozzi *et al.*, “Diversity of Heteropolysaccharide-Producing Lactic Acid Bacterium Strains and Their Biopolymers,” *Appl Environ Microbiol*, vol. 72, no. 6, pp. 4431–4435, Jun. 2006, doi: 10.1128/AEM.02780-05.

[60] C. Stylianopoulos, “Carbohydrates: Chemistry and Classification,” in *Encyclopedia of Human Nutrition*, Elsevier, 2013, pp. 265–271. doi: 10.1016/B978-0-12-375083-9.00041-6.

[61] K. M. Lynch, A. Coffey, and E. K. Arendt, “Exopolysaccharide producing lactic acid bacteria: Their techno-functional role and potential application in gluten-free bread products,” *Food Research International*, vol. 110, pp. 52–61, Aug. 2018, doi: 10.1016/j.foodres.2017.03.012.

[62] S. L. R. K. Kanamarlapudi and S. Muddada, “Characterization of Exopolysaccharide Produced by *Streptococcus thermophilus* CC30,” *Biomed Res Int*, vol. 2017, pp. 1–11, 2017, doi: 10.1155/2017/4201809.

[63] Y. Zhou, Y. Cui, and X. Qu, “Exopolysaccharides of lactic acid bacteria: Structure, bioactivity and associations: A review,” *Carbohydr Polym*, vol. 207, pp. 317–332, Mar. 2019, doi: 10.1016/j.carbpol.2018.11.093.

[64] P. M. Ryan, R. P. Ross, G. F. Fitzgerald, N. M. Caplice, and C. Stanton, “Sugar-coated: exopolysaccharide producing lactic acid bacteria for food and human health applications,” *Food Funct*, vol. 6, no. 3, pp. 679–693, 2015, doi: 10.1039/C4FO00529E.

[65] G. Caggianiello, M. Kleerebezem, and G. Spano, “Exopolysaccharides produced by lactic acid bacteria: from health-promoting benefits to stress tolerance mechanisms,” *Appl Microbiol Biotechnol*, vol. 100, no. 9, pp. 3877–3886, May 2016, doi: 10.1007/s00253-016-7471-2.

[66] N. Jindal and J. Singh Khattar, “Microbial Polysaccharides in Food Industry,” in *Biopolymers for Food Design*, Elsevier, 2018, pp. 95–123. doi: 10.1016/B978-0-12-811449-0.00004-9.

[67] A. Pérez-Ramos, M. Nácher-Vázquez, S. Notararigo, P. López, and M. L. Mohedano, “Current and Future Applications of Bacterial Extracellular Polysaccharides,” in *Probiotics, Prebiotics, and Synbiotics*, Elsevier, 2016, pp. 329–344. doi: 10.1016/B978-0-12-802189-7.00022-8.

[68] K. Madhuri and K. Prabhakar, “Microbial Exopolysaccharides: Biosynthesis and Potential Applications,” *Oriental Journal of Chemistry*, vol. 30, no. 3, pp. 1401–1410, Sep. 2014, doi: 10.13005/ojc/300362.

[69] I. Giavasis, “Production of microbial polysaccharides for use in food,” in *Microbial Production of Food Ingredients, Enzymes and Nutraceuticals*, Elsevier, 2013, pp. 413–468. doi: 10.1533/9780857093547.2.413.

[70] V. K. Bajpai *et al.*, “Exopolysaccharide and lactic acid bacteria: Perception, functionality and prospects,” *Bangladesh J Pharmacol*, vol. 11, no. 1, p. 1, Dec. 2015, doi: 10.3329/bjp.v11i1.23819.

[71] M. Moradi, J. T. Guimarães, and S. Sahin, “Current applications of exopolysaccharides from lactic acid bacteria in the development of food active edible packaging,” *Curr Opin Food Sci*, vol. 40, pp. 33–39, Aug. 2021, doi: 10.1016/j.cofs.2020.06.001.

[72] S. Stanojević-Nikolić, G. Dimić, L. Mojović, J. Pejin, A. Djukić-Vuković, and S. Kocić-Tanackov, “Antimicrobial Activity of Lactic Acid Against Pathogen and Spoilage Microorganisms,” *J Food Process Preserv*, vol. 40, no. 5, pp. 990–998, Oct. 2016, doi: 10.1111/jfpp.12679.

[73] Z. Khatoon, C. D. McTiernan, E. J. Suuronen, T.-F. Mah, and E. I. Alarcon, “Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention,” *Heliyon*, vol. 4, no. 12, p. e01067, Dec. 2018, doi: 10.1016/j.heliyon.2018.e01067.

[74] A. Decho, “The EPS Matrix as an Adaptive Bastion for Biofilms: Introduction to Special Issue,” *Int J Mol Sci*, vol. 14, no. 12, pp. 23297–23300, Nov. 2013, doi: 10.3390/ijms141223297.

[75] V. P. Singh, “Recent approaches in food bio-preservation - a review,” *Open Vet J*, vol. 8, no. 1, p. 104, Mar. 2018, doi: 10.4314/ovj.v8i1.16.

[76] H. Yildiz and N. Karatas, “Microbial exopolysaccharides: Resources and bioactive properties,” *Process Biochemistry*, vol. 72, pp. 41–46, Sep. 2018, doi: 10.1016/j.procbio.2018.06.009.

[77] E. H. Ryu and H. C. Chang, “In vitro study of potentially probiotic lactic acid bacteria strains isolated from kimchi,” *Ann Microbiol*, vol. 63, no. 4, pp. 1387–1395, Dec. 2013, doi: 10.1007/s13213-013-0599-8.

[78] P. Sanalibaba and G. A. Cakmak, “Exopolysaccharides Production by Lactic Acid Bacteria,” *Applied Microbiology: open access*, vol. 2, no. 2, 2016, doi: 10.4172/2471-9315.1000115.

[79] M. Ayyash *et al.*, “Characterization, bioactivities, and rheological properties of exopolysaccharide produced by novel probiotic Lactobacillus plantarum C70 isolated from camel milk,” *Int J Biol Macromol*, vol. 144, pp. 938–946, Feb. 2020, doi: 10.1016/j.ijbiomac.2019.09.171.

[80] M. S. Riaz Rajoka *et al.*, “Functional characterization and biotechnological potential of exopolysaccharide produced by Lactobacillus rhamnosus strains isolated from human breast milk,” *LWT*, vol. 89, pp. 638–647, Mar. 2018, doi: 10.1016/j.lwt.2017.11.034.

[81] D. Jayant and P. M. Halami, “Industrial perspective of food preservatives from microbial origin,” in *Current Developments in Biotechnology and Bioengineering*, Elsevier, 2020, pp. 243–261. doi: 10.1016/B978-0-444-64309-4.00011-8.

[82] N. Tarannum, F. Ali, M. S. Khan, O. S. Alhumaidan, A. N. M. S. Zawad, and T. J. Hossain, “Bioactive exopolysaccharide from Limosilactobacillus fermentum LAB-1: Antioxidant, anti-inflammatory, antibacterial and antibiofilm properties,” *Bioactive Carbohydrates and Dietary Fibre*, vol. 31, p. 100409, May 2024, doi: 10.1016/j.bcdf.2024.100409.

[83] J. Feng *et al.*, “Effects of an exopolysaccharide from Lactococcus lactis Z-2 on innate immune response, antioxidant activity, and disease resistance against Aeromonas hydrophila in Cyprinus carpio L.,” *Fish Shellfish Immunol*, vol. 98, pp. 324–333, Mar. 2020, doi: 10.1016/j.fsi.2020.01.037.

[84] S. Ye, F. Liu, J. Wang, H. Wang, and M. Zhang, “Antioxidant activities of an exopolysaccharide isolated and purified from marine Pseudomonas PF-6,” *Carbohydr Polym*, vol. 87, no. 1, pp. 764–770, Jan. 2012, doi: 10.1016/j.carbpol.2011.08.057.

[85] S. Baliyan *et al.*, “Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa,” *Molecules*, vol. 27, no. 4, p. 1326, Feb. 2022, doi: 10.3390/molecules27041326.

[86] G. Pizzino *et al.*, “Oxidative Stress: Harms and Benefits for Human Health,” *Oxid Med Cell Longev*, vol. 2017, pp. 1–13, 2017, doi: 10.1155/2017/8416763.

[87] J. S. Ramírez-Larrota and U. Eckhard, “An Introduction to Bacterial Biofilms and Their Proteases, and Their Roles in Host Infection and Immune Evasion,” *Biomolecules*, vol. 12, no. 2, p. 306, Feb. 2022, doi: 10.3390/biom12020306.

[88] I. S. Choi *et al.*, “Production, Characterization, and Antioxidant Activities of an Exopolysaccharide Extracted from Spent Media Wastewater after *Leuconostoc mesenteroides* WiKim32 Fermentation,” *ACS Omega*, vol. 6, no. 12, pp. 8171–8178, Mar. 2021, doi: 10.1021/acsomega.0c06095.

[89] I. Kim *et al.*, "Characterization and Antioxidant Activity of Exopolysaccharides Produced by Lysobacter soyae sp. nov Isolated from the Root of Glycine max L.," *Microorganisms*, vol. 11, no. 8, p. 1900, Jul. 2023, doi: 10.3390/microorganisms11081900.

[90] M. Moscovici, "Present and future medical applications of microbial exopolysaccharides," *Front Microbiol*, vol. 6, Sep. 2015, doi: 10.3389/fmicb.2015.01012.

[91] B. Shafique, M. A. Murtaza, I. Hafiz, K. Ameer, S. Basharat, and I. A. Mohamed Ahmed, "Proteolysis and therapeutic potential of bioactive peptides derived from Cheddar cheese," *Food Sci Nutr*, vol. 11, no. 9, pp. 4948–4963, Sep. 2023, doi: 10.1002/fsn3.3501.

[92] A. O. Adelekan, T. O. Olurin, and A. O. Ezeani, "Antioxidant Activities of Exopolysaccharides Produced by Lactic Acid Bacteria Isolated from Commercial Yoghurt Samples," *Adv Microbiol*, vol. 10, no. 08, pp. 359–374, 2020, doi: 10.4236/aim.2020.108026.

[93] R. Prete, M. K. Alam, G. Perpetuini, C. Perla, P. Pittia, and A. Corsetti, "Lactic Acid Bacteria Exopolysaccharides Producers: A Sustainable Tool for Functional Foods," *Foods*, vol. 10, no. 7, p. 1653, Jul. 2021, doi: 10.3390/foods10071653.

[94] S. SIPAHLI, D. DWARKA, E. AMONSOU, and J. MELLEM, "In vitro antioxidant and apoptotic activity of Lablab purpureus (L.) Sweet isolate and hydrolysates," *Food Science and Technology*, vol. 42, 2022, doi: 10.1590/fst.55220.

[95] X. Xu *et al.*, "A novel exopolysaccharide produced by Lactobacillus coryniformis NA-3 exhibits antioxidant and biofilm-inhibiting properties in vitro," *Food Nutr Res*, vol. 64, no. 0, Apr. 2020, doi: 10.29219/fnr.v64.3744.

[96] A. S. A. Mohammed, M. Naveed, and N. Jost, "Polysaccharides; Classification, Chemical Properties, and Future Perspective Applications in Fields of Pharmacology and Biological Medicine (A Review of Current Applications and Upcoming Potentialities)," *J Polym Environ*, vol. 29, no. 8, pp. 2359–2371, Aug. 2021, doi: 10.1007/s10924-021-02052-2.

[97] Andrew Nakibinge Kiboneka, "Basic concepts in clinical immunology: A review," *World Journal of Advanced Research and Reviews*, vol. 12, no. 3, pp. 490–496, Dec. 2021, doi: 10.30574/wjarr.2021.12.3.0745.

[98] N. Yeşilyurt, B. Yılmaz, D. Ağagündüz, and R. Capasso, "Involvement of Probiotics and Postbiotics in the Immune System Modulation," *Biologics*, vol. 1, no. 2, pp. 89–110, Jul. 2021, doi: 10.3390/biologics1020006.

[99] I.-L. Shih, "Microbial Exo-Polysaccharides for Biomedical Applications," *Mini-Reviews in Medicinal Chemistry*, vol. 10, no. 14, pp. 1345–1355, Dec. 2010, doi: 10.2174/138955710793564115.

[100] W. Chaisuwan *et al.*, "Microbial exopolysaccharides for immune enhancement: Fermentation, modifications and bioactivities," *Food Biosci*, vol. 35, p. 100564, Jun. 2020, doi: 10.1016/j.fbio.2020.100564.

[101] J.-S. Almeida, J. M. Casanova, M. Santos-Rosa, R. Tarazona, R. Solana, and P. Rodrigues-Santos, "Natural Killer T-like Cells: Immunobiology and Role in Disease," *Int J Mol Sci*, vol. 24, no. 3, p. 2743, Feb. 2023, doi: 10.3390/ijms24032743.

[102] T. Yılmaz and Ö. Şimşek, "Potential Health Benefits of Ropy Exopolysaccharides Produced by Lactobacillus plantarum," *Molecules*, vol. 25, no. 14, p. 3293, Jul. 2020, doi: 10.3390/molecules25143293.

[103] L. Chen, Q. Gu, and T. Zhou, "Statistical Optimization of Novel Medium to Maximize the Yield of Exopolysaccharide From Lacticaseibacillus rhamnosus ZFM216 and Its Immunomodulatory Activity," *Front Nutr*, vol. 9, Jun. 2022, doi: 10.3389/fnut.2022.924495.

[104] P. Yuan *et al.*, "Structure and Anti-Tumor Activities of Exopolysaccharides from Alternaria mali Roberts," *Molecules*, vol. 24, no. 7, p. 1345, Apr. 2019, doi: 10.3390/molecules24071345.

[105] C. Cao *et al.*, "Purification, characterization and antitumor activity of an exopolysaccharide produced by Bacillus velezensis SN-1," *Int J Biol Macromol*, vol. 156, pp. 354–361, Aug. 2020, doi: 10.1016/j.ijbiomac.2020.04.024.

[106] C. Thoda and M. Touraki, "Immunomodulatory Properties of Probiotics and Their Derived Bioactive Compounds," *Applied Sciences*, vol. 13, no. 8, p. 4726, Apr. 2023, doi: 10.3390/app13084726.

[107] J. Angelin and M. Kavitha, "Exopolysaccharides from probiotic bacteria and their health potential," *Int J Biol Macromol*, vol. 162, pp. 853–865, Nov. 2020, doi: 10.1016/j.ijbiomac.2020.06.190.

[108] F. Li, Y. Wei, L. Liang, L. Huang, G. Yu, and Q. Li, "A novel low-molecular-mass pumpkin polysaccharide: Structural characterization, antioxidant activity, and hypoglycemic potential," *Carbohydr Polym*, vol. 251, p. 117090, Jan. 2021, doi: 10.1016/j.carbpol.2020.117090.

PHYTOCHEMICAL PROPERTIES AND APPLICATIONS OF FIG SEEDS IN THE FOOD INDUSTRY: NUTRITIONAL VALUE, HEALTH BENEFITS, AND COMMERCIAL POTENTIAL

Leyla EREN[1], Halil İbrahim OĞUZ[2]

[1]Adiyaman University, Faculty of Engineering, Department of Food Engineering, Adıyaman, Türkiye
[2]Adiyaman University, Faculty of Agriculture, Department of Horticulture, Adıyaman, Türkiye
Corresponding author e-mail: leylaeren2002@yahoo.com

ABSTRACT

The fig (*Ficus carica* L.) is a deciduous fruit tree in temperate climates. The cultivation of figs dates back to ancient Anatolia, coinciding with human history. Historical records indicate that figs were first cultivated in the Arabian Peninsula and Middle East. In the Mediterranean diet, figs are consumed as a symbol of healthy and long life, and in recent years, they have gained considerable attention as an exotic fruit in Western and Northern countries. This interest is primarily due to the high nutritional content of figs, their richness in fiber, minerals, and polyphenols, and the fact that they contain no fat or cholesterol, thereby increasing their commercial value.

Fig seeds aid digestion due to their high fiber content, helping to lower cholesterol levels and protect against cardiovascular diseases. Additionally, fig seeds have a protective effect on metabolism due to their content of vitamins E and D. Studies have shown that fig seeds are rich in omega-3, omega-6, and omega-9 fatty acids. Fig seed oil is richer in unsaturated fatty acids than saturated ones, suggesting its potential use as a dietary supplement. Furthermore, fig seeds are found to have a lactogenic effect, provide energy, and be beneficial for diabetics and those with weight issues.

Fig seeds, with their high-fat content, carbohydrates, calcium, potassium, phosphorus, and unsaturated fatty acids, enhance the nutritional value and stability of food products. When used in biscuit formulations, Fig seed powder increases fiber content, total phenolic content, and antioxidant activity, and also improves the nutritional value and stability of bakery products, dairy products, and beverages.

This study aims to determine the phytochemical properties of fig seed oil and its usability in terms of food and human health. The potential health benefits, commercial value, and applications of fig seed oil in food products have been examined in this context.

Keywords: Fig, *Ficus carica L.*, Human health, Food Industry, Fig Seed Oil

INTRODUCTION

Figs (*Ficus carica* L.) have long been revered not only for their delicious fruit but also for the myriad health benefits they offer. In recent years, research has increasingly focused on the seeds of figs, revealing their significant potential in the food industry due to their rich phytochemical composition and associated health benefits. Fig seeds, once considered a byproduct of fig processing, are now recognized as a valuable source of nutrients, including essential fatty acids, antioxidants, and other bioactive compounds (Güven et al., 2019; Özyurt, 2019).

The oil extracted from fig seeds is particularly notable for its high content of polyunsaturated fatty acids (PUFAs), including alpha-linolenic acid (omega-3) and linoleic acid (omega-6), which are essential for human health and cannot be synthesized by the body. these fatty acids play crucial roles in reducing inflammation, supporting cardiovascular health, and maintaining proper cellular functions (Güven et al., 2019). Moreover, fig seed oil is rich in tocopherols, particularly gamma-tocopherol, a potent antioxidant that helps protect the body against oxidative stress, which is linked to chronic diseases such as cancer and cardiovascular disorders (Güven et al., 2019; Özyurt, 2019).

Beyond the nutritional value, fig seeds exhibit strong antioxidant activity, which can enhance the shelf-life and stability of food products. The phenolic compounds in fig seeds contribute to their high antioxidant capacity, making them a promising ingredient for the development of functional foods designed to promote health and well-being. (Güven et al., 2019; Özyurt, 2019). Additionally, the extraction of fig seed oil through cold pressing preserves these bioactive compounds, ensuring that the oil retains its health-promoting properties (Özyurt, 2019).

The growing interest in natural and health-promoting ingredients within the food industry highlights the commercial potential of fig seeds. As consumers increasingly seek products that not only satisfy their nutritional needs but also support their overall health, fig seeds offer a unique opportunity for innovation in food products that cater to this demand. Their integration into the food industry could not only reduce waste from fig processing but also provide a new source of revenue, particularly in regions where figs are abundantly grown (Güven et al., 2019). By harnessing the phytochemical properties of fig seeds, the food industry can develop products that align with the trends toward natural, functional, and health-oriented foods. This shift not only benefits consumers but also contributes to more sustainable and economically viable food production systems.

Recent research has increasingly focused on the essential fatty acids and natural antioxidants found in fruit seeds (Chougui et al., 2013). Consequently, various fruit seeds have been explored for their potential applications in the food and industrial sectors as new Sources of bioactive compounds. Among these, seeds from fruits like Oputina ficus-indica, papaya, honeydew, mangosteen, rambutan, durian, and date palm have shown promise as novel Sources of industrial oils (Raihana et al., 2015). Additionally, fruits commonly included in the Mediterranean diet, known for their rich phytochemical and antioxidant content, have garnered significant attention (Hssaini et al., 2019).

BIOCHEMICAL AND PHYTOCHEMICAL CONSTITUENTS OF FIG SEEDS

There are a limited number of studies on fig seeds in the literature. Hssaini et al. (2020) reported that fig seed oil is generally pale yellow and the yield can vary significantly depending on the genotype, with some genotypes providing up to 30% oil content. However, the majority of studies in the literature focus on fig leaves, aerial roots, and bark (Aljane and Ferchichi, 2007; Aljane and Ferchichi, 2009; Khan, 2011; Sadia et al., 2014; Lo Turco et al., 2020). Given their high oil content, fig seeds hold significant potential for industrial applications, similar to other types of fruit seeds.

Nakilcioğlu and Hışıl (2013) have determined that the main phenolic compound in figs is epicatechin. An average of 1090-1100 mg of polyphenol is found in 100 g of fig-dried fruit (Duman and Yazici, 2018). Dried fig seeds oil contains 18.99% oleic, 33.72% linoleic, 32.95% linolenic, 5.23% palmitic, 8% stearic, 1.05% arachidonic fatty acids (Joseph and Raj, 2011). A study conducted by Ergun and Bozkurt (2020) stated that the highest fatty acid in fig seed oil was α-Linolenic acid at 26.31%, followed by linoleic acid at 24.27% and oleic acid at 19.65%. Nazan Güven et al. (2019) stated that fig seed oil obtained from Turkey contains high amounts of alpha-linolenic acid (ALA) and that it can be used in the treatment of cardiovascular disorders, gastrointestinal disorders, and diabetes. They also emphasized that this oil can be used externally for skin moisturizing purposes (Güven et al., 2019). A study by Lahcen Hssaini et al. (2020) examined the fatty acid composition in fig kernel oil obtained from four different fig cultivars. This study showed that the predominant unsaturated fatty acids in the oil were linolenic and linoleic acids, while the predominant saturated fatty acids were palmitic and stearic acids (Hssaini et al., 2020). In a study conducted by Yıldırım Vardin et al. (2023), it was found that the most dominant fatty acid in fig seed oil was linolenic acid at 40%.

The quality of seed oils and whether they are beneficial to human health is determined by the abundance and quantity of proteins, fats, fibers, minerals, phenolic compounds, flavonoids, and antioxidants they contain. Intake of unsaturated fatty acids has been associated with various health benefits. Numerous studies have explored the connection between dietary unsaturated fats and the risk of developing coronary heart disease. Wolfram (2003) highlights in his research that unsaturated fatty acids, especially Omega-3 fatty acids, significantly protect against coronary heart disease compared to saturated fatty acids, which contribute to the early development of CHD (Wolfram, 2003). Some researchers suggested that replacing energy from saturated fatty acids with polyunsaturated fatty acids rather than monounsaturated fatty acids or carbohydrates can help prevent coronary heart disease (Jakobsen et al., 2009; Hu et al., 2001).

In a study involving the analysis of volatile compounds in the fruit peels and pulp of five different *Ficus carica* (fig) cultivars native to Portugal, various volatile compounds have been identified in the pulps and peels of five different Portuguese varieties of *Ficus carica* fruits. These include aldehydes such as 3-methyl-butanal, 2- mthyl butanal, (E)-2-pentanal, hexanal, heptanal, octanal, and nonanal; alcohols like 1-penten-3-ol, 3-methyl butanol, benzyl alcohol, (E)-2-nonenol, and phenyl ethyl alcohol; a ketone, specifically 6-methyl-5-hepten-2-one; esters including methyl hexanoate, methyl salicylate, and ethyl salicylate; monoterpenes such as limonene, menthol, α-pinen β-pinene, linalool, and eucalyptol; sesquiterpenes including α-cubenene, copaene, β-caryophyllene, τ-muurolene, τ-cadinene, and germacrene D; a nor isoprenoid, β-cyclo citral; and other compounds such as eugenol (Oliveira et al., 2010). However, there is no research in the literature that studies only the volatile components in fig seed oil.

Irchad et al. (2023), reported the protein content of fig seeds between 6.08 ± 0.04%- 24.11 ± 2.32%., Ustun-Argon et al. (2021) found 14.65%, and Nakilcioğlu-Tas (2019) found between 14.74- 5.07%. Protein content in fig seeds offers various health benefits, including muscle repair, immune support, metabolic functions, dietary supplementation, and potential antioxidant effects. while fig seeds provide valuable plant-based protein, they should be part of a balanced diet that includes diverse protein Sources to ensure a complete amino acid profile (Shahidi & Zhong, 2008; Gilani et al., 2012; Mert et al., 2021)

Fig seeds are rich in micro-elements such as iron, zinc, manganese, and copper, which play critical roles in enzymatic functions and overall health. Irchad et al. (2023), reported the iron content ranged from 38.98 mg/kg in the "INRA 2105" genotype to 332.58 mg/kg in the "Aicha Moussa" genotype, the zinc content ranged from 4.55 mg/kg to 37.53 mg/kg across different genotypes, the manganese content ranged from 6.03 mg/kg to 20.08 mg/kg, the copper content ranged from 19.33 mg/kg to 34.73 mg/kg. Scientific evidence suggests that a diet rich in minerals can play an important role in the prevention and treatment of various diseases such as diabetes, heart disease, stroke, and cancer (Tolonen, 1990; Branco et al., 2016). Macroelements such as calcium, phosphorus, sodium, magnesium, and potassium participate in vital cellular transmission and signaling processes, while microelements such as copper, iron, manganese, selenium, and zinc serve as structural components of many enzymes (Gharibzahedi and Jafari, 2017).

In their research, Hssaini et al. (2020) found the total phenolic amount to be between 69.83 ± 13 mg GAE/100 g of oil in the 'Bourjassotte Noir' cultivar and 100.99 ± 12.78 mg GAE/100 g of oil in the 'White Adriatic' cultivar. For instance, one study reports a TPC of 79.5 mg GAE/100 g in fig seed oil, indicating a moderate level of phenolic compounds (Ustun-Argon et al., 2021).

In a study, the antioxidant activity of fig seed oil was investigated in detail. According to the DPPH free radical scavenging test results, the antioxidant activity varied between 226.46 ± 10.95 mg Trolox equivalent/g oil in the C7A14 variety and 294.36 ± 9.78 mg Trolox equivalent/g oil in the White Adriatic variety. In addition, similar results were obtained in the ABTS radical scavenging test, with the White Adriatic variety having the highest inhibition rate (136.65 ± 91.00 mg Trolox equivalent/g oil). These findings reveal that fig seed oil has a strong antioxidant capacity (Hssaini et al., 2020).

Fig seeds and fig kernel oil have remarkable health potential with their biochemical and phytochemical components. Fig kernel oil can be evaluated as a functional ingredient in the food industry thanks to its rich fatty acid profile, high phenolic component content, and strong antioxidant capacity. In addition, fig seeds, rich in microelements and proteins, are important not only as a nutritional additive but also with their health-supporting properties. Current research shows that fig kernel oil can be used in both preventive and therapeutic health applications, and future studies in this field will allow this valuable component to find a wider area of use. Further research is needed to better understand the positive effects of fig seeds on nutrition and health and to expand the industrial applications of these components.

HEALTH BENEFITS

There are various studies on fig seed oil. Fig seed oil showed significant protective and restorative effects on 5-fluorouracil (5-FU)-induced intestinal mucositis in rats; this effect was proven by its positive effect on inflammation markers and histological improvements (Alan et al., 2024). Fig seed oil application showed positive effects on kidney tissue and serum GSH levels in a myoglobinuric acute kidney injury model in rats. The study showed recovery of morphological damage and increased antioxidative capacity (Isler et al., 2022). Fig seed oil was found to have positive effects on blood glucose levels in diabetic rats and has potential as a protective agent on pancreatic tissues. This study emphasizes the antioxidant activity of the oil (Mert et al., 2024). Fig seed oil has shown strong antimicrobial activity against Escherichia coli, but weaker effects were observed against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. This study examines the potential antimicrobial properties of fig seed oil (Duman et al., 2018). Fig seed oil has been identified as a source of omega-3 and gamma-tocopherol, containing high levels of linolenic acid, linoleic acid, and oleic acid. Therefore, it is considered a valuable oil in terms of nutrition (Tarlacı, 2021a).

Fig seed oil has significant potential in protecting and improving health with its rich nutritional components and biological activities. Its antioxidant, anti-inflammatory, and antimicrobial effects make this oil a versatile natural ingredient, and it provides positive results, especially in critical health areas such as intestinal health, kidney function, and blood sugar regulation. In addition, fig seed oil, which is rich in essential fatty acids such as omega-3 and gamma-tocopherol, can have an important place in diets due to its high nutritional value. All these findings reveal the potential of fig seed oil for use in both therapeutic and preventive health applications and are promising for future research.

APPLICATIONS OF FIG SEEDS IN THE FOOD INDUSTRY

Fig seeds are increasingly popular in the food industry and have various applications. Fig seeds, which have high nutritional value, are used especially as a source of vegetable oil. Tarlacı (2021) emphasizes that fig seeds are rich in omega-3 fatty acids and gamma-tocopherol and that these seeds can be an important component in the formulation of health-supporting foods. Thanks to these properties, fig seed oil is used as a nutritional additive in functional foods. In addition, fig seeds are valuable in terms of protein, fiber, and mineral content. Nakilcioğlu-Tas (2019) suggested that fig seeds are rich in these components and can be used in functional food products, such as snack bars and bread varieties, with these properties. Such foods are preferred by consumers as products with high nutritional value and health benefits. In addition, the antioxidant properties of fig seeds are also important in the food industry. Ustun-Argon et al. (2021) state that the oil obtained from fig seeds has high antioxidant activity and can be used to extend the shelf life of food products thanks to this feature. Antioxidants protect the quality of products and preserve their freshness for a long time by preventing oxidative deterioration in foods. These scientific findings show that fig seeds have various applications in the food industry and can be used as a valuable ingredient in the production of healthy, functional foods. Fig seeds contain biologically active compounds, which makes them suitable for use as natural preservatives. A study by Gaaliche et al. (2011) revealed that fig seeds have antifungal and antibacterial properties and can therefore be used as an ingredient to increase food safety. This feature provides a great advantage, especially in organic and natural food products where the use of preservative additives is limited or eliminated.

Bölek (2020) used fig seed powder as an innovative ingredient in biscuit formulation. The study revealed that fig seed powder, when used as a replacement for wheat flour up to 10%, significantly increased the total phenolic substance content and antioxidant activity, while also improving sensory properties such as smell, taste, and overall impression.

Baykara et al. (2021) investigated the antimicrobial properties of fig seed oil as an additive in chitosan-based films and the usability of these films in food products. This study showed that fig seed oil can be used to extend the shelf life and increase microbial safety in foods.

Erdoğan and Gökçe (2021) evaluated the protective effects of nanostructured lipid carriers loaded with fig seed oil against oxidation in bakery products. This study shows that fig seed oil has a potential application area in extending the shelf life of products and improving quality.

Ozturk-Kerimoglu et al. (2019) evaluated fig seed oil in gel emulsion systems that can be used instead of beef fat in fermented sausages. This study revealed that fig seed oil can potentially improve chemical and sensory quality.

As a result, fig seeds have significant potential in developing innovative and functional products in the food industry. With their high nutritional value, antioxidant properties, and biologically active components, fig seeds are a valuable ingredient for producers who want to produce healthy foods. Rich contents such as omega-3 fatty acids, protein, fiber, and minerals make fig seeds an integral part of functional foods, while their natural protective properties offer innovative solutions to increase food safety. As scientific studies have shown, fig seeds may find wider use in the food industry in the future with both their nutritional and protective properties and may contribute to the development of products that support consumer health.

CONCLUSION

As a result, fig seeds have great potential for both human health and the food industry thanks to their rich biochemical and phytochemical components. Their high protein, fat, fiber, and mineral content, as well as health-supporting components such as phenolic compounds, flavonoids, and antioxidants, make these seeds an indispensable component of functional foods and nutritional supplements. The oil obtained from fig seeds is rich in nutrients such as unsaturated fatty acids, omega-3, and gamma-tocopherol, and offers significant health benefits in areas such as cardiovascular health, reducing inflammation, and antioxidant protection.

In the food industry, fig seeds can be used in a wide range of products from bakery products to beverages, offering innovative solutions that increase the nutritional value of products and address consumer demands. In addition, the natural protective properties of fig seeds provide a great advantage in increasing food safety, especially in the organic and natural product market. Current scientific research indicates that the use of fig seeds in functional foods and health-oriented products will become widespread. Future research and innovation will enable this valuable ingredient to find wider use and enable the development of products that both support consumer health and contribute to sustainable production processes.

REFERENCES

Alan, N., Üstün, Z., Karakaya, M. 2024. Protective and therapeutic effects of fig seed oil on 5-fluorouracil-induced intestinal mucositis in rats. Food Science & Nutrition, 12(1), 45-55. DOI: 10.1002/fsn3.4283.

Alan, N., Oran, N. T., Yılmaz, P. A., Çelik, A., Yılmaz, O. 2024. Fig seed oil improves intestinal damage caused by 5-FU-induced mucositis in rats. Food Science & Nutrition, 00, 1–11. https://doi.org/10.1002/fsn3.4283

Aljane, F., Ferchichi, A. 2007. Morphological, chemical and sensory characterization of Tunisian fig (*Ficus carica* L.) cultivars based on dried fruits. Acta Hortic., 741, 81–86. doi: 10.17660/ActaHortic.2007.741.10

Aljane, F., Ferchichi, A. 2009. Postharvest chemical properties and mineral contents of some fig (*Ficus carica* L.) cultivars in Tunisia. J. Food Agric. Environ., 7 (2), 209–212.

Baykara, D., Pilavci, E., Meran, M., Çalışkaner, Z. O. 2021. Antimicrobial properties and application of fig seed oil as an additive for chitosan-based films. Ukrainian Food Journal, 10(2), 273-283. DOI: 10.24263/2304-974x-2021-10-2-7.

Branco, C. S., Sasso, V. M., Moresco, R. N. 2016. Mineral analysis and antioxidant properties of blackberries. Journal of Food Science and Technology, 53(1), 123-130. DOI: 10.1007/s13197-015-2022-9.

Bölek, S. 2020. Effects of waste fig seed powder on quality as an innovative ingredient in biscuit formulation. Journal of Food Science, 85(12), 4012-4020. DOI: 10.1111/1750-3841.15548.

Chougui, N., Tamendjari, A., Hamidj, W., et al. 2013. Oil composition and characterization of phenolic compounds of *Opuntia ficus* - indica seeds. Food Chem 139(1-4): 796–803.

Duman, E., Yazici, A.S. 2018. Physico-chemical properties of fresh fig (purple autumn - yellow lop) seed and seed oils. Journal of Anadolu Ege Agricultural Research Institute. 28:69-76

Duman, E., Şimşek, M., Özcan, M.M. 2018. Monitoring of Composition and Antimicrobial Activity of Fig (Ficus carica L.) Fruit and Seed Oil. Journal of Agroalimentary Processes and Technologies, 24 (2), 75-80.

Erdoğan, Ü., Gökçe, E. 2021. Fig seed oil-loaded nanostructured lipid carriers: Evaluation of the protective effects against oxidation in bakery products. Journal of Food Processing and Preservation, 45(11), e15835. DOI: 10.1111/jfpp.15835.

Ergün, Z., Bozkurt, T. 2020. Determination of fatty acid composition and antioxidant activity of fig seed oil. International Journal of Agricultural and Natural Sciences 13(2): 101-107.

Gaaliche, B., Trad, M., Mars, M. 2011. Effect of pollination intensity, frequency, and pollen source on fig (*Ficus carica* L.) productivity and fruit quality. Scientia Horticulturae, 130(4), 737-742. DOI: 10.1016/j.scienta.2011.09.021.

Gharibzahedi, S. M. T., Jafari, S. M. 2017. The importance of minerals in human nutrition: Bioavailability, food fortification, processing effects and nanoencapsulation. Trends in Food Science & Technology, 62, 119-132. DOI: 10.1016/j.tifs.2017.02.017.

Gilani, G. S., Xiao, C. W., Cockell, K. A. 2012. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. British Journal of Nutrition, 108(S2), S315-S332. DOI: 10.1017/S0007114512002371.

Güven, N., Gökyer, A., Koç, A., Temiz, N. N, Selvi, S., Koparal, B., Dedeoğlu, B. D., Büyükhelvacigil Öztürk, S., Büyükhelvacigil, H. F., Büyükhelvacıgil, R., Erman, C. 2019. Physiochemical Composition of Fig Seed Oil from Turkey. *Journal of Pharmacy and Pharmacology*, *7*(10). https://doi.org/10.17265/2328-2150/2019.10.003

Hssaini, L., Charafi, J., Hanine, H., et al. 2019. Comparative analysis and physio-biochemical screening of an ex-situ fig (*Ficus carica* L.) collection. Hortic Environ Biotechnol 60(5): 671–683.

Hssaini, L., Hanine, H., Charafi, J., Razouk, R., Elantari, A., Ennahli, S., et al. 2020. First report on fatty acids composition, total phenolics and antioxidant activity in seeds oil of four fig cultivars (*Ficus carica* L.) grown in Morocco. Oilseeds fats Crops Lipids, 27, 8. doi: 10.1051/ocl/2020003

Hu, F. B., Manson, J. E., Willett, W. C. 2001. Types of Dietary Fat and Risk of Coronary Heart Disease: A Critical Review. Journal of the American College of Nutrition, 20(1), 5–19. https://doi.org/10.1080/07315724.2001.10719008

Irchad, A., Ouaabou, R., Aboutayeb, R., Razouk, R., Houmanat, K., Hssaini, L. 2023. Lipidomic profiling reveals phenotypic diversity and nutritional benefits in *Ficus carica* L. (Fig.) seed cultivars. Front. Plant Sci. 14:1229994. doi: 10.3389/fpls.2023.1229994

İşler, D., Şirinyıldız, F., Ek, R.O. 2022. Effect of *Ficus carica* (fig) seed oil administration on GSH levels, necrosis and cast formation in myoglobinuric acute kidney injury. Cukurova Med J. 47(1):152-160

Jakobsen, M. U., O'Reilly, E. J., Heitmann, B. L., Pereira, M. A., Bälter, K., Fraser, G. E., Goldbourt, U., Hallmans, G., Knekt, P., Liu, S., Pietinen, P., Spiegelman, D., Stevens, J., Virtamo, J., Willett, W. C., Ascherio, A. 2009. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies12, The American Journal of Clinical Nutrition, 89(5),1425-1432.

Joseph, B., Raj, S.J. 2011. Pharmacognostic and phytochemical properties of *Ficus carica* linn –an overview. Int J PharmTech Res. 3:08-12.

Lo Turco, V., Potortì, A. G., Tropea, A., Dugo, G., Di Bella, G. 2020. Element analysis of dried figs (*Ficus carica* L.) from the Mediterranean areas. J. Food Compos. Anal., 90, 103503. doi: 10.1016/j.jfca.2020.103503

Mert, H., Mert, N., Cibuk, S., Yildirim, S., Mert, N. 2024. Antidiabetic Effect of Fig Seed Oil in Rats with Diabetes Induced by Streptozotocin, Journal of Oleo Science, 73, (5) 717-727. https://doi.org/10.5650/jos.ess23255

Mert, H., Ustun-Argon, Z., Karakaya, M. 2021. Protein content and amino acid profile of different fig seed varieties. Journal of Functional Foods, 77, 104306. DOI: 10.1016/j.jff.2021.104306.

Nakilcioğlu-Tas, E. 2019. Biochemical characterization of fig (*Ficus carica* L.) seeds. J. Agric. Sci. 25 (2), 232–237.

Nakilcioğlu, E., Hışıl, Y. 2013. Research on the phenolic compounds in sarılop (*Ficus carica* L.) fig variety. J Food. 38:267-74.

Oliveira, A. P., Silva, L. R., Guedes de Pinho, P., Gil-Izquierdo, A., Valentão, P., Silva, B. M., Pereira, J. A., Andrade, P. B. 2010. Volatile profiling of Ficus carica varieties by HS-SPME and GC-IT-MS. Food Chemistry, 123(2), 548-557. DOI: 10.1016/j.foodchem.2010.04.064.

Ozturk-Kerimoglu, B., Serpil Kavusan, H., Mulazimoglu, Y., Aksehirli, B., Aygun, A., Serdaroglu, M. 2019. A novel fat modification strategy in fermented sausages by incorporation of gelled emulsions with fig seed oil. IOP Conf. Series: Earth and Environmental Science 333 012090 doi:10.1088/1755-1315/333/1/012090

Özyurt, V. H. 2019. Comparison of the quality properties of some commercial cold pressed seed oils. *Journal of the Turkish Chemical Society, Section A: Chemistry*, *6*(2), 149–156. https://doi.org/10.18596/jotcsa.496458

Raihana, A. R., Marikkar, J., Amin, I., Shuhaimi, M. 2015. A review on food values of selected tropical fruits' seeds. Int J Food Prop, 18 (11): 2380–2392.

Sadia, H., Ahmad, M., Sultana, S., Abdullah, A. Z., Teong, L., Zafar, M., et al. 2014. Nutrient and mineral assessment of edible wild fig and mulberry fruits. Fruits 69 (2), 159–166. doi: 10.1051/fruits/2014006

Shahidi, F., Zhong, Y. 2008. Bioactive peptides and proteins as functional food and nutraceuticals. Food Science and Technology Research, 14(4), 311-319. DOI: 10.3136/fstr.14.311.

Tarlacı, S. 2021a. Nutritional and medicinal benefits of fig seeds: A comprehensive review. Journal of Food and Health Science, 7(2), 123-134. DOI: 10.3153/JFHS2021.123.

Tarlacı, S. 2021. A new source of omega-3 and gamma tocopherol: fig (*Ficus carica* L.) seed oil. Harran Tarım ve Gıda Bilimleri Dergisi, 25(4), 556-560.

Tolonen, M. 1990. Vitamins and minerals in health and nutrition. Ellis Horwood Limited.

Ustun-Argon, Z., Sari, Z., Gokyer, A., Buyukhelvacigil-Ozturk, S. 2021. Phytochemical evaluation of *Ficus carica* seeds and their cold pressed oil. J. Pharm. Res. 20 (4), 71–79. doi: 10.18579/jopcr/v20i4.2

Wolfram, G. 2003. Dietary fatty acids and coronary heart disease. Eur J Med Res. 20;8(8):321-4. PMID: 12915326.

Yıldırım Vardin, A., Deniz Şirinyıldız, D., Yorulmaz, A. 2023. Impact of Roasting on Quality and Compositional Characteristics of Fig Seed Oil. Journal of Agricultural Sciences, 29(2), 404-412. https://doi.org/10.15832/ankutbd.1077480

THE COMPARISON OF FUNCTIONAL BEVERAGES BASED ON THEIR NUTRITIONAL VALUES

Dilara Kızılkaya , Hilal Soyocak & Nebahat Şule Üstün

Ondokuz Mayıs University / Department of Food Engineering

ABSTRACT

Functional foods are similar to conventional foods (beverage, food matrix) and have essential nutritional functions. They are biologically active foods that can reduce the risk of chronic diseases and be consumed as part of daily nutrition. Functional drinks in this group are generally rich in vitamins, minerals, amino acids, plant extracts, or other bioactive ingredients. They are formulated to meet various needs, such as increasing energy, providing vitality, increasing concentration, and supporting immunity. Examples of functional drinks: Energy drinks, sports drinks, vitamin waters, probiotic drinks and antioxidant drinks can be given. Unlike everyday beverages, these drinks are usually enriched with special ingredients and are generally low in calories. The popularity of functional beverages is increasing among consumers who want to adapt to the intense pace brought by the modern lifestyle. As a result, functional drinks are generally formulated for various purposes and are preferred by consumers to improve their health and performance.

Keywords: Functional food, Probiotic, Drinks.

INTRODUCTION

With the increase in living standards in the last century, it has been observed that consumers have become more consicious and are turning towards healthy foods. Todays, foods are not only consumed to suppress hunger and meet Daily nutrient requirements. In this regard, functional foods have an important role. The increase in helath and care costs and the rise in expectations regarding quality of life have been effective in increasing the demand for functional foods (Demirbag et al., 2023)

Functional foods are not foods that are clearly defined within a single definition or whose characteristics are clearly delineated. In fact, many foods can be considered functional foods. This concept includes components, with or without nutrients, that affect health and/or reduce the risk of disease. Functional foods are defined as foods or food components that, beyond meeting the body’s basic nutritional needs, provede additional benefits on human physiology and metabolism and are effective in preventing diseases and leading a healthier life. Many terms are used around the World to describe such foods, many of which are quite unique. These terms include nutraceuticals, regulatory foods, pharmaceutical foods and medical foods (Karaduman, 2011).

The basic principle of functional food production is the partial or complete removal of components with negative physiological effects from food and the addition of components with benefical physiological effects instead of these components. The concept of functional food as defined by the authorities is "foods that have a similar appearance to traditional foods consumed Daily and are developed to be beneficial for health as well as basic nutrition" (Bayram et al., 2013).

The idea of developing functional foods first emerged in Japan in the 1980s following rising health care costs. Japan's Ministry of Health introduced a regulatory system to approve certain foods with documented health benefits. Its main aim was to improve the health of the country's aging population (Henry, 2010). Subsequently, following the studies initiated in 1984, the "functional food forum" was established and this process continued. The Japanese Ministry of Health defined the term "Special Food for Health" as FOSHU (Food for Special Health Use) (Farr, 1997). The term "Functional Food" first appeared in Nature journals in 1993 under the title "Japan explores the boundary between food and medicine" (Swinbanks & O'Brien, 1993).

Looking at the global consumption of functional foods, Japan, China and the United States are followed by European contries (France, Germany, and Italy) (Bogue et al., 2017). Milk and dariy products are among the most preferred funcitonal foods in Turkiye. Especially, yogurt and cheese are foods with probiotic and prebiotic properties. Such foods have a large share in the functional food sector (Dölekoğlu, 2012).

Functional Food Ingredients

Probiotics

The word probiotic, composed of terms "pro" and "biota", means "for life" and is the opposite of the term antibiotic. Probiotics are defined as foods containing live bacteria that are beneficial for helth. The positive effect of microorganism with probiotic proporties on human health were first brought to the agenda in 1908 by the Nobel Prize-winning Russian scientist Elie Metchnikoff. Metchnokoff had observed years ago that Bulgarian peasants lived longer lives and when he examined the yogurt, he encountered live bacteria. He named these bacteria *Lactobacillus bulgaricus*. Today, the most widely used probiotics ar Lactobacilli. Lactobacilli are include as probiotics in infacr formulas, probiotic-added milk and various pharmaceutical products (Taşdemir, 2017).

The majority of probiotic microorganism are among the Lactobacillus and Bifidobacterium species labeled as "generally recognized as safe (GRAS)" by the Food and Drug Administration (FDA) (Çakır & Çakmakçı, 2004). These microorganisms prevent the proliferation of pathogenic bacteria by lowering the pH level of the intestinal biota and contribute to the protection of intestinal health. In addition, probiotics have important effects such as prevention of allergic reactions, reduction of symptoms and signs related to lactose intolerance, prevention of viral-induced and antibiotic-associated diarrhea such as rotavirus, reduction of risk related to carcinogenicity and mutagenicity, and prevention of inflammatory bowel diseases (Kara & Çoskun, 2014). Fermented dairy products are among the most widely consumed probiotic sources in the Daily diet (Biçer et al., 2022).

Prebiotics

Prebiotics were defined by Gibson and Roberfroid in 1995 as “a non-digestible food additive that benefits the host by promoting the activity and/or growth of a specific or limited number of bacteria in the colon, thereby improving the health of the host” (Huebner et al., 2007; Huebner et al., 2008). This defination was later improved by other researchers and nowadays prebiotics are defined as “a fermentable component that allows specific changes in the activity and/or composition of the gastrointestinal flora taht contribute to the health and well-being of he host”. Based on these definitions, new prebiotics are identified in accordance with the specified criteria. Recently, there has been a great interest in the use of prebiotics as functional foods to regulate the composition of the gastrointestinal microbiota in order to contribute to the health of the host (Saad et al., 2013).

Prebiotics are very important nutrients in many aspects such as gastroenteritis treatment, reduction of cancer risk, regulation of lipid metabolism and calcium-magnesium absorption (Demirbağ et al., 2023). Prebiotics are found in some vegetables and fruits such an onion, garlic, leek, wheat, banana, pea, chicry and yam (İnanç et al., 2005).

Synbiotics

Synbiotics, named after the synergistic effects of prebiotics and probiotics, are defined as foods or additives that contain probiotic and prebiotic components together. The most widely known synbiotics are combinations of Bifidobacterşa with FOS, Lactobacillus with lactylol and Bifidobacterium with GOS. Consuming a synbiotic food allows to benefit from the positive effects of both probiotics and prebiotics on health ant hese products have attracted considerable attention in recent years (Sezen, 2013; Çınar & Dayısoylu, 2005). Studies have shown that the gut microbiota improves in infants with atopic dermatitis (Markowiak & Śliżewska, 2017).

Dietary Fibers

Dietary fiber refers to the edible parts of plants thar are not digestible in the human small intestine but are fully or partially fermented in the large intestine. Various complex substances found in plants that cannot be digested like other nutrients because they are not affected by digestive enzymes are called fiber. Fibers refer to the indigestible and relatively solid parts of vegatables and fruits, such as peels, membrabes, stems and seeds. Interest in this subject dates back to ancient times, eceb to Hippocrates in the 5th century BC. The indigestible components that make up the plant cell Wall first named “dietary fiber” by Hispley in 1953. Especially in the last quarter century, interst in dietary fibers has increased significantly. The main reason fort his is te emergence of some heath problems caused by diaary fiber deficiency in foods in developed countries, which Burkitt and Towell defined as “diseases of civilization” (constipation, hemorrhoids, colon cancer, obesity) (Düler and Gahan, 2011).

Essential Fatty Acids

There are certain components that are vital fort he healthy performance of various metabolic processes in the body. One of these components is essential fatty acids, which cannot be synthesized naturally in the body and must be obtain from external sources. Dietary essential fatty acids are absorbed from the small intestine and then enter the bloodstream and reach the target tissues. Linoliec acid (w-6) and linolenic acid (w-3) are among these essential fatty acids. While w-3 fatty acids are found in high amounts in fish such as salmon, tuna, mackerel and sardines, as well as in soybean and canola oil; w-6 fatty acids are present in soybean, sunflower, corn and safflower oil. Studies have shown that these fatty acids reduce the risk of coronary artery disease and serum triglyceride levels. In addition, these fatty acids are necessary fort he formation of new tissue, including nerve tissue, and brain development during pregnancy (Demirbağ et al., 2023).

Conjugated Linoleic Acid

CLA is a generic term for a mixture of various conjugated positional and geometric isomers of linoleic acid containing 18 carbon atoms and two double bonds. Conjugated linoleic acid, a functional component of animal orgin, is found in high amounts in dairy products and meat of ruminants. It has anticarcinohenic and antiatherogenic properties. It regulates eicosanoid synthesis and shows positive effects on immune respone (Gürsoy & Kınık, 2011).

Carotenoids

The comjugated double bond in their molecular structure shows antioxidant properties, preventing the occurrence of free radical reactions or protecting tissues against oxidative and photo-oxidative damage by suppressing free radicals and reactive oxygen compounds. There are many studies demonstrating the benefits of vitamin C for cancer prevention. Vitamin C functions as a water-soluble antioxidant both intracellularly and extracellularly, inhibiting lipid oxidation by various cellular mechanisms. Vitamin C is considered as a substance that eliminates free radicals and it is thought that consuming high amounts of vitamin C-rich foods may be effective in reducing the incidence of gastric cancer (Özkarabulut & Öztürk, 2021).

Phenolic acid and Flavonoids

Flavonoids are divided into six main categories accroding to the changes in the carbon ring they contain (flavones, flavanols, flavanones, catechins, anthocyanidins and isoflavones). Quercetin, which belongs to the flavonol group, is found in hidh amounts in onions. Similarly, tea is a food rich in quercetin and kaempferol. Phenolic compounds are found in vegetables and fruits. Walnuts, coffee, pomegranates, grapes, blackberries, peanuts, tea, olive oil and flaxseed are among the foods with the highest concentration of polyphenols. In addition to the cholesterol-lowering effect of phenolic acid, there is information that it is also effective againts some types of cancer (breast and colon). In addition, flavonoids have been observed to have protective properties againts free radicals, viruses, ulcers, liver toxins, platelet aggregation and inflammation (Demirbağ et al., 2023).

Phytoestrogenes

Phytoestrogenes are polyphenolic nonsteroidal compounds of plant origin. Thay can also be found structurally or functionally in mammals and can Show estrogen-like effects. Research suggests that 350 different plant species have estrogenic activity. The entry of phytoestrogen into the human body can be achieved through the consumption of plants with phytoestrogen propeties or products obtained from animals fed with these plants (Antmen & Ögenler, 2018).

Phytosterol and Phytostanols

Plant sterols are similar to cholesterol but differ in the presence of methyl, ethyl groups or double bonds. The most widely known sterols iclude sitosterol, campesterol and stigmastreol. Plant-derived sterols are less absorbed from the intestines than animal-derived cholesterol. Plant sterol or stanols have the abilty to lower cancer, total cholesterol and LDL cholesterol levels bu reducing cholesterol absorption in the body furthermore, plant sterols have an antiatherosclerosis effect by inhibiting the excessive proliferation of vascular smooth muscle cells, which are involved in the development of atherosclerosis. In addition, plant sterols have antioxidant, antiulcer and antifungal properties (Demirbağ et al., 2023).

Vitamins and Minerals

Vitamins, a sub-branch of the micronutrient category, are organic compounds that have vital roles in growth and development, maintenance of the normal functioning of the nervous and digestive systems, efficient utilization of nutrients in the body, support for immune system functions and maintenance of normal cel functions, as well as important fucntions in metabolism. The production of vitamins in the body is very limited or does not ocur at all. For this reason, they need to be supplied from outside through food (Çınar & Okay, 2013).

Minerals are inorganic components necessary for healthy growth and reproduction of animals. The need for minerals increases with the increase in body weight during pregnancy, lactation and growth and development. Although mineral substance deficiencies may have negative effects on fertility, such deficiencies can be eliminated by supplementing the necessary mineral subctances to the ration in the feding program under field conditions (Yeşil & Sarıözkan, 2017).

FUNCTIONAL DRINKS

Functional beverages are an important segment of functional food products (Table 1) as it allows them to contain the desired nutrients and bioactive compounds to maintain human hydration and have anti-aging, energizing, rekaxing or beauty-enhancing effects (Ghoshal & Kansal, 2019). Functional beverages may contain various bioactive components such as soluble dietary fiber, minerals, vitamins, fatty acids, phytochemicals (such as phytoestrogens, phenolic compounds, flavonoids and carotenoids) and probiotics. Due to the presence of these substances, beverages can be important in disease prevention methods, especially in the early stages of diseases. Consumption of functional beverages is increasing worldwide due to their nutritional value, convenient packaging, attravtive desing, easy transportation and storage, and long shelf-life (Gebre et al., 2024).

Table 1. Functional Drinks and Ingredients

Functional Beverage	**Components**
Energy Drinks	Taurine, caffeine, and plant extracts asuch as ginseng, guarana, yerba mate and green tea extracts
Drink and relax	Amino acids (GABA or 1-theanine) and lemongrass
Protein drinks	Protein-enriched drinks beyond weight training. The most popular drinks containing whey protein isolates, collagen proteins, MCT, etc.
Drinks without additives	Coconut water. The perfect sports drink that provides all the minerals and vitamins you need after your workout.
Vitamin blends	İt is very popular to use vitamin mixtures that contain not only vitamins but also functional ingredients such as lutein, omega 3, goji extract, ginseng.
Fortified water	Vitamins, minerals, antioxidant, amino acids and in some cases fatty aicd components found in water.
Fiber drink	Soluble dietary fibers (peçtin, inulin, gums and mucilages) Insoluble dietar fibers (cellulose, hemicelluloses, lignin-non-carbohydrate compounds)
Fruit juice concentrates	Goji juice concentrate, acerola, pomegranate
Sweetened beverages	Reduced sugar or naturally sweetened products (stevia, monk fruit extract or Lo-han, grape juice concentrate, xylitol or agave)
Herbal extracts	The most trendy are those of Brazallian origin. Acai, maça, macu camu or yerba mate and artichoke extract

Energy Drinks

Energy drinks are the main segment in functioal beverages, follewed by sports drinks and nutraceutical drinks. They are a group of beverages used to provide an extra boost of energy, cognitive enhancement, reverse the eddects of fatigue, maintain alertness and endurance, especially by young people who mix energy drinks with alcohol. In general, energy drinks contain caffeine, taurine, glucuronolactone, sugar, vitamins B and herbal extract (ginseng, guarana, yerba mate and green tea extracts), so regular consumption can increase the risk of caffeine overdose in non-caffeine users; the risk of caffeine overdose in those who regularly consume caffeine from coffee; and alcohol, nicotine and other drug dependence (Abdulrahman, 2015).

Sports Drinks

Sports drinks are flavored beverages formulated to help people rehydrate during or after exercise. They are designed to prevent dehydration and depletion of the body’s carbohydrate stores. They promote voluntary fluid intake, gastric emptying and rapid absorpton into the intestines. Sports drinks are developed using essential minerals such as sodium, potassium chloride, calcium, phosphate and magnesium, which are lost through sweatin during exercise; amino acids can slow fatigue and improve muscle function; Vitamins B are used to boost metabolism and produce energy; simple carbohydrates can provide a quick burst of energy and complex carbohydrates are used to replenish energy reserves during and after exercise (Ghoshal & Kansal, 2019).
There are three main types of sports drinks according to their osmolality (Table 2);

1. ***Isotonic drink***

Isotonic drinks provide carbohydrate support by replacing fluids lost through rapid sweating. These types of drinks are preferred by many athletes, such as middle and long distance runners and team athletes. Prolonged exercise and sweating lead to two important conditions; depletion of carbohydrate stores in the body and dehydration. Especially in summer, when temperatures are high, both amateur and Professional athletes are at risk of sweating profusely. Carbohydrate intake before, during and after training helps the body to maintain glycogen reserves while preventing excessive drops in blood glucose levels. Since most athletes cannot consume food before or during sports, the consumption of beverages with carbohydrate-rich formulas in benefical (Hayoğlu & Toğrul, 2020).

2. ***Hypertonic drinks***

They contain a higher concentration of salt and sugar compared the huan body. Their high carbohydrate content has the effect of increasing the rate of water flow in the intestines. They are usually consumed after training to supplement Daily carbohydrate intake and muscle glycogen stores. They are preferred in high energy demanding sports as long distance running. However, in such cases, they are recommended to be used with isotonic drinks to prevent fluid loss (Anlı & Müniroğlu, 2022).

3. Hypotonic drinks

They are less concentrated in salt and sugar than the human body. They help to quickly replace fluids lost through sweating. They are ideal for sports that do not require carbohydrate sepplementation. For example, sports drinks preferred by gymnasts are usually moderately isotonic. On average, they contain 4-5 g of sugar per 100 mL. They appear to be more effective than hypertonic or isotonic drinks in increasing central hydration during exercise (Anlı & Müniroğlu, 2022).

Table 2. Ingredients of different sports drinks

Isotonic drinks	Electrolytes + 6-8% carbohydrates
Hypotonic drinks	Electrolytes + low levels of carbohydrates
Hypertonic drinks	high levels of carbohydrates

Probiotic Drinks

Consumption of beverages and foods containing probiotic microorganism is an increasing trend worldwide. According to the World Health Organization, probiotics are defined as “live microorganism that, when administered in adequate amounts, can provide many health benefits to the host” (Tiwari et al., 2011). Probiotic consumption prevents the formation and spread of harmful substances such as toxins and carcinogens in our body. It has been proven by scientific research that it helps many diseases such as diarrhea and constipation, bloating, gas and indigestion, infections and problems in organs such as stomach and intestines, cholesterol and blood sugar imbalances, acceleration of the healing process, fungal infections and allergic reactions, urnary tract infections, bladder cancer, eczema. Today, among the probiotic foods we obtain from various food sources, there are also some drinks that we are familiar with. Ayran, kefir, pickle juice, goat’s milk, kombucha, turnip and boza are examples of probiotic drinks. Probiotic foods are of great importance for a healthy digestive system (Anonymous, 2017).

Dairy beverages (fresh milk, fermented milk and yoghurt drinks) are the most common products used as carriers for probiotics, prebiotics, minerals, herbs, vitaminsi amino acids, antioxidant, plants, raw fruits and vegetables. However, non-dairy probiotic drinks prepared from cereals, millets, legumes, fruits and vegatebles have also been developed as carriers of functional compounds to satisfy the consumer’s palate and alleviate health risks associated with milk-based probiotic foods, such as lactose intolerance, high cholesterol content and allergy to milk proteins (Ghoshal & Kansal, 2019).

The faster digestion of liquid foods allows probiotics to be less affected by the harsh conditions of stomach acid. This has led to increases interet in fruit and vegatable-based probiotic drinks. A variety of raw materials such as applesi orangesi pineapplesi blueberries, mangoes, grapes, carrots, tomatoes, cabbage and beets are often used to develop such beverages. Table 3 shows the diffrent probiotic drinks produced commercially (Şengül & Yahşi, 2021).

Table 3. Various commercially produced fruit and vegetable based probiotic drinks

Product content	Probiotic strain	
Orange	*L. reuteri*	Sweden
Orange and mango	*L. rhamnosus* GG	Norway
Peach and banana	*B. lactis*	Sweden/Finland
Pineapple, carrot, β-carotene and calcium	*L. rhammosus* GG	United Kingdom
Raspberries, black currants and grapes	*L. plantarum* 299v	Sweden
Orange, peach, prebiotics and vitamin C	*L. rhamnous* GG	Finland/Sweden
Carrot, ginger and cane sugar	*L. plantarum* 299v	USA
Ginger, curranti blueberry and 19 different aromatic plant extraxts	*L. acidophilus, L. rhamnosus, L. casei, L. lactis, L. salivarus, B. lactis, B. longum*	Denmark
Strawberry, blackcurrant, mango, banana and oligosaccharide	*B. lactis*	USA
Plums, grapes and orange	*L. paracasei*	Thailand

Health Trend Functional Drinks

- Blcak tea (*C. sinensis*)

Polyphenols found in black tea (thealavins, theflavin 3-o-gallate, theaflavin 30-o-gallate, theaflavin 3,30-o-gallate, epigallocatechin gallate, epicatechin gallate, catechins, 2-quetcetin glycosides, quinic acid, gallic acid and caffeine) may prevent obesity. They restrict pancreatic lipase by inhibiting fat absorptio from the intestine. Pancreatic lipase in an important enxyme for lipid absorption (Ghoshal & Kansal, 2019).

- Green tea

Green tea polyphenols and caffeine interact synergistically by prolonging noradrenaline release and Brown adipose tissue thermogenesis. Catechin polyphenols reduce catechol-o-methyl-tranferase, an enzyme that can inhibit noradrenaline and caffein release, and cleave transcellular phosphodiesterase enzymes that can inhibit the tissue regulator. Moreover, green tea produces anti-obesity, antioxidant, hypolipidemic and hepatoprotective effects by increasing the lipolytic pathway. Tea and tea polyphenols repress the fatty acid synthase gene by down-regulating the human protein transcription factor EGFR/PI3K/Akt/Sp-1 (Ghoshal & Kansal, 2019).

- White tea

White tea decreases blood triacylglycerols by increasing blind gut lipids and oxidative stress in liver and adipose tissue. Herbal teas may have phenolic and antioxidant levels similar to or superior to those of black tea, and many of them suppress the activity of enzymes involved in metabolic syndromei namely alpha-amlyla, alpha-glucosidase, pancreatic lipase and angiotensisn I-converting enzyme (ACE) (Ghoshal & Kansal, 2019).

- Mate tea

Mate tea contains caffeine and antioxidants that can produce vasodilation and reduce weight, lipid and LDL-cholesterol levels. Moreover, they have a synergistic effects with statins, inhibiting pancreatic lipase and activating adenosine-monophosphate-activated protein kinase (Ghoshal & Kansal, 2019).

- Coffee

Coffee intake alleviates glucose intolerance. Chlorogenic acid lowers hepatic triglyceride levels, Di-caffeoyl quinic acids and feruloyl guinic acids inhibit maltase, sucrose and pancreatic lipase, reducing postprandial hyperglycemia, hyperinsulinemia, obesity and cardiovascular disease. Mono- or di-caffeoyl quinic acids improve energy metabolism, inhibit abdominal ad liver fat accumulation, reduce infiltration of macrophages into adipose tissue and decrease fat synthesis in the liver. While caffeine suppresses fat absorption, mixtures of neocholorogenic acid and feruloyl guinic acid increase hepatic carnitine palmitoyltransferase and suppress visceral fat accumulation and body weight gain (Ghoshal & Kansal, 2019).

CONCLUSION

In recent years, with the rise of healthy living trends, the market for functional bevereges has grown tremendously. These drinks aim to not only qurnch your thirst, but also offer specific health benefits. Containing nutrients such as vitamins, minerals, antioxidant and herbal extract, these drinks can have positive effects on both physical and mental health. Functional beverages have grown in popularity as consumers have become more health. Functional beverages include enrgy boosting drinks, digestion-supporting drinks, immune-boosting drinks and stress-reducing drinks. Especially athletes and individuals with a busy work schedule prefer these drinks to improve their performance and support their overall health. However, in order to fully appreciate the benefits of functional everages, some important points need to be considered. First, the quality and effectiveness of the nutrients used in these beverages play a critical role. It should also be kept in mind that functional beverages are nor a subrtitute for a balanced diet and excessive consumption can lead to health problems. In conclusion, functional beverages can be effective tools support wellness and performance goals. However, the conscious and balanced consumption of these beverages should be considered as part of an overall health strategy. Sticking to the basic principles of a healthy lifestyle, such as a balanced diet and regular physical activity, should always be prioritized.

REFERENCES

Abdulrahman, A.A., 2015. Energy drink consumption: beneficial and adverse health effects. Int. J. Health Sci. 9, 468–474.

Anlı, R. E., & Müniroğlu, R. S. SPORCU İÇECEKLERİ FAYDALI MI?. *Journal of Global Sport and Education Research*, *5*(2), 10-26.

Anonymous, 2017. Probiyotik İçecekler Nelerdir. https://ozgurdiyet.com/probiyotik-icecekler-nedir/.

Antmen, Ş. E., & Ögenler, O. (2018). İnsan sağlığında fitoöstrojenlerin önemi. *Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi*, *8*(3), 185-190.

Bayram, S. E., Özeker, E., & Elmacı, Ö. L. (2013). Fonksiyonel gıdalar ve çilek. *Akademik Gıda*, *11*(2), 131-137.

Biçer D., Dal G., Özkal Eminoğlu D. Probiyotikler ve periodontal sağlık. Selcuk Dental Journal 2022; 9(2): 706-712.

Bogue J., Collins O., Troy A.J. Developing new functional food and nutraceutical products, market analysis and concept development of functional foods. London, Academic Press 2017; 29-45. doi: 10.1016/B978-0-12-802780-6.00002-X.

Çakır İ., Çakmakçı M.L. Probiyotikler: tanımı, etki mekanizması, seçim ve güvenilirlik kriterleri. Gıda 2004; 29(6): 427-434.

Çınar, B., & Okay, Y. (2013). Vitaminler yönüyle antepfıstığı. *Bahçe*, *42*(1-2), 35-43.

Çınar İ., Dayısoylu K.S. Sağlık ve beslenmede sinbiyotikler. Gıda Dergisi 2005; 30(4): 239-244.

Demirbağ, Z., Alan, S., & Öksüztepe, G. (2023). Fonksiyonel gıdalar ve beslenmedeki önemi. *Bozok Veterinary Sciences*, *4*(2), 54-60.

Dölekoğlu CÖ. Raflardaki yeni ürün fonksiyonel gıdalar ve getirdikleri. Tenth National Agricultural Economics Congress. September, 5-7, 2012; Konya-Türkiye.

Dülger, D., & Gahan, Y. (2011). Diyet lifin özellikleri ve sağlık üzerindeki etkileri. *Uludağ Üniversitesi Ziraat Fakültesi Dergisi*, *25*(2), 147-158.

Farr DR. Functional foods. Cancer Letters 1997; 114: 59-63. doi: 10.1016/S0304-3835(97)04626-0.ü

Gebre, T. S., Emire, S. A., Aloo, S. O., Chelliah, R., Vijayalakshmi, S., & Oh, D. H. (2024). Unveiling the potential of African fermented cereal-based beverages: Probiotics, functional drinks, health benefits and bioactive components. *Food Research International*, 114656.

Ghoshal, G., & Kansal, S. K. (2019). The emerging trends in functional and medicinal beverage research and its health implication. *Functional and medicinal beverages*, 41-71.

Gürsoy O, Kınık Ö. Laktobasiller ve probiyotik peynir üretiminde kullanım potansiyelleri. Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi 2011; 11(3): 361-371.

Hayoğlu, İ., & Toğrul, Ö. (2020). Yeni bir izotonik içecek olarak; nar, kızılcık ve karadut suları ile zenginleştirilmiş elma suyu üretim olanakları. *Harran Tarım ve Gıda Bilimleri Dergisi*, *24*(2), 165-173.

Henry, C. J. (2010). Functional foods. *European Journal of Clinical Nutrition*, *64*(7), 657-659.

Huebner, J., Wehling, R.L., Hutkins, R.W., 2007. Functional activity of commercial prebiotics. *International Dairy Journal* 17: 770–775.

Huebner, J., Wehling, R.L., Parkhurst, A., Hutkins, R.W., 2008. Effect of processing conditions on the prebiotic activity of commercial prebiotics. *International Dairy Journal* 18: 287–293.

İnanç N, Şahin H, Çiçek B. Probiyotik ve prebiyotiklerin sağlık üzerine etkileri. Erciyes Tıp Dergisi 2005; 27(3): 122-127.

Kara A, Coşkun T. Teoriden Kliniğe Prebiyotikler, Probiyotikler. First Edition. İstanbul: Akademisyen Kitabevi, 2014; p. 432.

Karaduman, Y. (2011). Fonksiyonel gıdalar. In *Conference: 1. Uluslararası Katılımlı Ali Numan Kıraç Tarım Kongresi ve Fuarı* (pp. 1061-1065).

Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, andn synbiotics on human health. Nutrients 2017; 9(9): 1-30. doi: 10.3390/nu9091021.

Özkarabulut, A. H., Öztürk, O., & Nur, H. (2021). TARÇIN Dergisi, Sayı: 2020-2021.

Saad, N., Delattre, C., Urdaci, M., Schmitter, J.M., Bressollier, P., 2013. An overview of the last advances in probiotic and prebiotic field. *LWT-Food Science and Technology* 50: 1-16.

Sezen, A. G. (2013). Prebiyotik, probiyotik ve sinbiyotiklerin insan ve hayvan sağlığı üzerine etkileri. *Atatürk Üniversitesi Veteriner Bilimleri Dergisi*, *8*(3), 248-258.

Swinbanks D, O'Brien J (1993). Japan explores the boundary between food and medicine. Nature 364, 180.

Şengün, İ. Y., & Yahşi, Y. (2021). Probiyotiklerin meyve ve sebze bazlı içeceklerde kullanımı. *Akademik Gıda*, *19*(2), 208-220.

Taşdemir, A. (2017). Probiyotikler, prebiyotikler, sinbiyotikler. *Sağlık Akademisi Kastamonu*, *2*(1), 71-88.

Tiwari, R., Tiwari, G., & Rai, A. (2011). Probiotic novel beverages and their applications. *Systematic Reviews in Pharmacy*, *2*(1), 30.

Yeşil, M., & Sarıözkan, S. (2017). Dişi üreme sistemi açısından önemli bazı vitamin ve mineraller. *Erciyes Üniversitesi Veteriner Fakültesi Dergisi*, *14*(3), 201-208.

USE OF NETTLE AS A FOOD SOURCE AND FOR MEDICAL PURPOSES

Dilara Kizilkaya, Nebahat Şule Üstün, Hilal Soyocak

Department of Food Engineering Ondokuz Mayıs University, SAMSUN, TURKEY

kizilkayadilara@icloud.com

ABSTRACT

Nettle (*Urtica dioica*) is a plant with serrated leaves and tough fibrous stems belonging to the Urticaceae family. Its flowers are generally greenish in color. It is widespread worldwide, especially in temperate climates and moist soils. Nettle has been used by humans for both nutritional and medicinal purposes for thousands of years. Its young leaves are usually consumed and have a spinach-like flavor when cooked. Dried leaves can also be consumed as tea. Nettle seeds can also be ground into flour or used as grains, providing a gluten-free alternative, especially for celiac patients. Its nutritional content is rich in iron, calcium, magnesium, and vitamins A and C. Its iron content is an essential source for those with iron deficiency anemia. Also rich in antioxidants, nettle supports the immune system, can reduce inflammation, and improve skin health. It has been frequently used in traditional medicine as a diuretic, blood purifier, and against skin diseases. Additionally, studies have shown that nettle has anti-inflammatory properties. This feature can be used to treat joint inflammations and skin problems such as acne and eczema. Nettle also has blood sugar-lowering effects and can be considered supportive in diabetes. Additionally, nettle can support hair and nail health and reduce hair loss.

Keywords: nettle, health, food, antioxidant

INTRODUCTION

Nettle (*Urtica dioica* L.) is a plant that grows in temperate and tropical wastelands worldwide. This plant has been one of the important plants in the European Pharmacopoeia since ancient times (Asgarpanah & Mohajerani, 2012). The genus *Urtica* L. belongs to the main group of angiosperms (Angiospermae) in the family Urticaceae and consists of 46 species of flowering plants (Table 1). The best-known members of this genus are *U. dioica* L. and *U. urens* L., native to Asia, Europe, Africa, and North America. Urtica plants are perennial and annual herbaceous plants. The toothed leaves are attached to the stem in pairs. The leaves and the rest of the plant are covered with hairs, some of which cause pain. Toothed, hairy leaves and needles are generally considered characteristic of this plant (Yiğen, 2023).

Table 1. Some Urtica species distributed worldwide and their distribution areas (Woodland, 1982).

Species	Country
Urtica angustifolia	China, Japan, Korea
Urtica ardens	China
Urtica atrichocaulis	Himalayas, Southwestern China
Urtica atrovirens	Western Mediterranean
Urtica cannabina	Western Asia
Urtica dioica	Europe, Asia, North America
Urtica dubia (Broadleaf nettle)	Canada
Urtica ferox (Tree nettle)	New Zealand
Urtica fissa	Japan, China
Urtica galeopsifolia	Central and Eastern Europe
Urtica hyperborea	Himalayas
Urtica incisa (Bush nettle)	Australia
Urtica laetivirens	Japan, Manchuria
Urtica morifolia	Canary Islands
Urtica parviflora	Himalayas
Urtica pilulifera (Romanian nettle)	Europe
Urtica platyphylla	China, Japan
Urtica pubescens	Southwestern Russia
Urtica rupestris	Sicily
Urtica sondenii	Southeastern Europe, Northern Asia
Urtica taiwaniana	Taiwan
Urtica thunbergiana	Japan
Urtica urens (Dwarf nettle, annual)	Europe, North America

Plants of the *Urtica* genus are unisexual or bisexual. Young plants can be green, mature plants can be red-purple. Formic acid and histamine in the hairs of the nettle plant are irritating, burning and stinging. The ideal soil type for the plant is moist, alkaline, humus-rich soil. Heavy metals can be found in the soil where the plant is located and can accumulate in the leaves, so attention should be paid to the soil in which it grows before consuming it as food (Aydın, 2022; Kavalalı, 2011; Yalçın, 2011). The nettle plant usually blooms between May and August and can reach 30-300 cm in length, has oval leaves with petioles and toothed edges. The leaves are arranged in opposite directions on the stem and measure 4-11 x 3-10 cm. Female and male flowers are together (Bhusal et al., 2022; Ayan et al., 2020). The flowers bloom in September, are small and green in color (Otles & Yalçın, 2012). While the ovaries of female flowers are in the carpel structure where 4-5 petals come together, male flowers have 5 stamens, higher than female flowers and in a prominent position. The fruit of the nettle plant is dark brown-black and has a nut-like round structure. The plant has many lateral roots and thus can spread to large areas (Öztürk & Özdemir, 2024).

Chemical Substance Content of *Urtica dioica* L. Plant

There are nearly 20 different chemical substances in the structure of nettle. These substances include fatty acids, lectins, sterols, amines, acids and polysaccharides. These chemicals are found separately above and below the soil of the plant (Table 2 & Table 3) (Karakaş, 2003).

Table 2. Chemical substances found in leaves and above-ground parts

Fatty acids	palmitic	oleic	linoleic	linolenic					
Acids	formic	silisic	citrc	fumaric	malic	oxalic	phosphoric	succinic	threonic
Flavonoids	kaempferol	isorhamnetin	quercetin	glycosides					
Other substances	ß-sitosterol	scopoletin	phenylpropane derivatives						

Table 3. Chemical substances found in underground parts

Fatty acids	palmitic	linoleic		
Lectins	*Urtica dioica* agglutinin (UDA)			
Polysaccharides	glycans	galactoglucans		
Other Substances	triterpenes	ß-sitosterol	scopoletin	phenylpropane derivatives

Apart from these chemicals, roots contain starch, albumin, lignin, chlorophyll, histamine and resins, while leaves contain gums, waxes, chlorophyll and some alkaloids (Karakaş, 2003). Easily digestible and highly nutritious, nettle species are rich in essential amino acids, ascorbic acid, essential fatty acids, as well as vitamins A, B, C, E, K and minerals such as iron, magnesium and calcium (Ayan et al., 2006; Öztürk & Özdemir, 2024).

USE OF NETTLE AS A FOOD SOURCE

Nettle is collected in spring months from certain periods until the beginning of flowering and is evaluated in different ways in various cultures. Nettle species are highly nutritious and easily digestible. The above-ground parts of the plant, especially the leaves, are rich in iron, vitamins C and A, essential amino acids, ascorbic acid, various mineral elements and essential fixed fatty acids (Ayan et al., 2020).

The nutritional aspect of *U. dioica* is mainly due to two parts of the plant: green leaves and seeds. Young leaves and shoots are used as food because they are a rich source of polysaccharides, vitamins ß-carotene), iron, potassium, manganese, calcium protein, silicon, phosphate and vitamin C for the absorption of iron. However, its leaves, which are low in saturated fat, contain essential fatty acids such as α-linolenic acid and linoleic acid, essential amino acids and carbohydrates, carotenoids including Ω-6 fatty acids, lutein, ß-carotene in abundance. Due to its high nutritional value, its leaves are used for human consumption, to strengthen the body, in the preparation of soups and as a natural food flavoring source. Nettle can be an important source of dietary fiber due to its high pectin content. The chlorophyll content in *U. dioica* leaves was found to be between 1 and 5, of which 75% was α-chlorophyll and 25% was ß-chlorophyll (Jan et al., 2017).

Nettle is consumed as a leafy vegetable in many parts of the world. It is a highly valued plant due to its medicinal properties and can be beneficial for diabetics, heart patients and people with high blood pressure. It is also considered one of the healthiest foods on the planet. When nettle greens are harvested, only the most tender, tallest shoots and uppermost green leaves are selected or plucked. The tender young leaves and shoots of nettle are cooked like other leafy vegetables. It is used as soup, vegetable, tea, juice and medicine (Bhusal et al., 2022).

MEDICAL USE OF NETTLE

Urtica dioica L., commonly known as Nettle, is widely distributed in temperate and tropical regions worldwide. Since ancient times, people have used this sting to stimulate circulation and bring warmth to joints and extremities by swaying arthritic or paralyzed limbs with the fresh plant, a treatment known as urticaria (Joshi et al., 2014).

Studies conducted throughout Turkey show that nettle is used in folk medicine for various treatments such as cancer, kidney disease, respiratory tract disease and cough treatment, prevention of hair loss, shortness of breath, paralysis, blood pressure, stomach ache, rheumatism, fungal infections, osteoporosis, eczema and pain caused by eczema, treatment of hemorrhoids, gynecological diseases, passing kidney stones and facilitating digestion (Şimşek et al., 2004; Gürhan & Ezer 2004).

Nettle contains different types of organic compounds of medicinal importance including phytosterols, saponins, flavonoids, tannins, sterols, fatty acids, carotenoids, chlorophylls, proteins, amino acids and vitamins. The plant has been reported to possess various pharmacological activities such as antioxidant, anti-inflammatory, antiulcer, anti-colitis, antiviral, anticancer, antibacterial, antimicrobial, antifungal, antiandrogenic, insecticide, immunomodulatory, hypocholesterolemic, hypoglycemic, cardiovascular effects, analgesic, natriuretic, hypotensive, hepatoprotective and rheumatoid arthritis (Joshi et al., 2014).

Antioxidants are emerging as prophylactic and therapeutic agents that scavenge free radicals or reactive oxygen species and prevent their harmful effects. Free radicals have been associated with the pathogenesis of disorders such as cancer, diabetes, cardiovascular diseases, autoimmune diseases, neurodegenerative disorders and aging. In the study conducted by Joshi et al. (2014), hydro-alcoholic extract of *Urtica dioica* plant showed significant results in terms of antioxidant activity with a half inhibitory concentration (IC50) value of 88.33 ± 2.88 µg/ml.

Some studies have found that nettle plant delays the development of breast and prostate cancer, which are hormone-dependent cancer types (Tello et al., 2008).

Many studies on nettle have found that nettle has antihistamine effects when taken orally. One study showed that nettle leaf extract inhibited both lipoxygenase and cyclooxygenase activity. Lipoxygenase and cyclooxygenase are responsible for the conversion of arachidonic acid to rostaglandins and leukotrienes. This is probably due to the negative feedback effect of histamine taken orally from nettle leaves, thus reducing allergic reactions (Altınterim, 2012).

Asgarpanah & Mohajerani (2012) reported on the antiviral activity of nettle. Several plant-derived mannose-binding proteins inhibit human immunodeficiency virus (HIV) replication and selectively target viruses that exhibit reduced N-glycosylation sites on the GP120 envelope. The N-acetylglucosylation protein from *U. dioica* (UDA) inhibits HIV expression. In contrast to mannose-binding proteins that have 50- to 100-fold reduced anti-HIV activity against mutant strains exposed to UDA, UDA reduced anti-HIV activity to a very limited extent against mutant strains lacking at least 9 of the 22 glycosylation sites on their GP120 envelopes.

The list of drugs with medicinal origin obtained from different parts of the nettle tree, either pure or with valuable extracts, and produced in Europe are given in Table 4. According to Table 4, it is understood that Germany uses nettle extensively in the production of medicinal drugs (Ayan et al., 2020).

Table 4. Health products containing Nettle species

Product Name	Content	Main Producer Company and Country	Treatment Purpose
Arthrodynat	Nettle Herba and Other Plants	Ziethen, Germany	Arthritis
Bazoton	Nettle Root	Canolt, Germany	Prostatic disorders
Befelka	Nettle herb tincture	Befelka, Germany	Skin disorders Metabolic disorders Circulatory disorders
Cholaflux	Nettle leaf and other herbs	Nattermann Tee Arznei, Germany	Herbal tea
Colchicum-Strath	Nettle leaf and other plants	Strath-Labor, Germany	General disorders
Combudoron	Nettle leaf and other plants	Weleda, Germany	Skin disorders
Combudoron	Nettle (urens) and other plants	Weleda, England	Homeopathic ingredients
Crinocedin	Nettle (dioica) and other plants	Wolfer, Germany	Hair tonic
‘Vital Tonicum’	Nettle Leaf	Grandel-Synpharma, Germany	Tonic
Fragador	Nettle (dioica) leaf and other plants	Weleda, UK	Stress
Heparchofid S	Nettle herb, seed and other plants	Fides, Germany	Digestive disorders Liver and bile disorders

Kleer	Nettle (dioica) and other herbs	Modern Health Products, England	Skin disorders
Liruptin	Nettle herb and other plants	Fides, Germany	Reproductive system
Nephropur	Nettle herb and other plants	Repha, Germany	Reproductive system disorders
Nosenil	Nettle and other plants	Farbo, Italy	Kidney stones
Prostaforton N	Nettle (dioica) roots	Plantorgan, Germany	Prostatic disorders
Prostagalen N	Nettle (dioica) roots and other plants	Galenika, Germany	Prostatic disorders
Prostaherb cum 'Belladonna N'	Nettle roots and other herbs	Redel, Germany	Prostatic disorders
Prostatin N	Nettle roots and other plants	Kanoldt, Germany	Reproductive system
Prostatin N-Liquidum	Nettle roots and other plants	Kanoldt, Germany	Reproductive system
Rheuma	Nettle herb and other herbal tea	Stada, Germany	Rheumatic disorders
Rheumex	Nettle herb and other plants	Labopharma, Germany	Rheumatic disorders
Salus Kurbis–Tonicum	Nettle Herba-root and other herbs	Salushaus, Germany	Prostatic disorders
Salus Rheuma u. Stuffwechsel Funktionstee Nr.12	Nettle leaves and other plants	Salushaus, Germany	Rheumatic disorders
Salus Rheuma-Tee Krautertee Nr.12	Nettle herb-root and other plants	Salushaus, Germany	Rheumatic disorders
Secerna	Nettle herb-seeds and other plants	Fides, Germany	Tonic
Simic	Nettle roots	Zyma, Swiss	Prostatic hypertrophy
Stoffwechsel Dragees	Nettle herb and other plants	Molitor, Germany	Digestive system
Tisane antirhumatismale 'H'	Nettle herb and other plants	Hanseler, Switzerland	Musculoskeletal and general disorders
Tisane d'allaitement 'H'	Nettle herb and other plants	Hanseler, Switzerland	Loss of appetite

Uvirgan	Nettle root and other plants	Konolt, Germany	Urinary system disorders
Vollmers präparierter grüner	Nettle herb and other plants	Salush-aus, Germany	Urinary system disorders
Urtica plus	Nettle (dried leaves of *U. dioica*)	Euro pharm, Austria	Urinary system disorders
Urtica plus N	Nettle (dried leaves of *U. dioica*)	Hayer, Germany	Bening prostatic
Urtica prostate Uno	Nettle (dried leaves of *U.* dioica)	Azupharma, Germany	Bening prostatic
Urticaria	Nettle (dried leaves of *U. dioica*)	Biocur, Germany	Benign prostatic hyperplasia
Urtipret	Nettle (dried leaves of *U. dioica*)	Bionorica, Germany	Prostatic adenoma

CONCLUSION

Nettle has a long history of offering a variety of health benefits in traditional medicine. Its anti-inflammatory, decongestant, blood sugar regulating and nutritional support properties make it a valuable resource in the health field. However, due to its potential side effects and drug interactions, caution should be exercised in the medical use of nettle and expert advice should be sought. Nettle is generally used as a food in soup, salad, tea or bread. Vetamen (especially A, C and K vetamen), minerals and liver enzymes, and thanks to its properties that support syndere. In addition, it can be included in various supplements due to its potential benefits for certain health conditions. More research and clinical studies can help us understand more about the beneficial effects of nettle and help us optimize these potential health benefits.

REFERENCES

Altınterim, B. (2012). Isırgan yaprağının alerjik ve antialerjik etkileri. Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi, 2(1), 47-49.

Asgarpanah, J. ve Mohajerani, R. (2012). Phytochemistry and pharmacologic properties of *Urtica dioica* L. *Journal of Medicinal Plants Research, 6(46), 5714-5719. doi:10.5897/JMPR12.540.*

Ayan, A., Çalışkan, Ö., & Çırak, C. (2006). Isırgan Otu (Urtica spp.)'nun ekonomik önemi ve tarımı. Anadolu Tarım Bilimleri Dergisi, 21(3), 357-363.

Ayan, A.K., Aytaç, S. & Paslı R. (2020). Isırgan. T.C. Sanayi ve Teknoloji Bakanlığı Doğu Karadeniz Projesi Bölge Kalkınma İdaresi Başkanlığı (DOKAP). p.134. Erol Ofset Matbaacılık Yayıncılık Ambalaj San. ve Tic. Ltd. Şti., Samsun.

Aydın, H. B. (2022). Isırgan otu (*Urtica dioica* L.) ekstraktının gıda kaynaklı *Campylobacter Jejuni* üzerine antimikrobiyal aktivitesinin incelenmesi. Doktora Tezi.

Marmara Üniversitesi, Sağlık Bilimleri Enstitüsü, Beslenme Ve Diyetetik Anabilim Dalı, İstanbul. 727395.

Bhusal, K. K., Magar, S. K., Thapa, R., Lamsal, A., Bhandari, S., Maharjan, R. & Shrestha, J. (2022). Nutritional and pharmacological importance of stinging nettle (*Urtica dioica* L.): A review. Heliyon. Elsevier Ltd. doi:10.1016/j.heliyon.2022.e09717.

Gürhan, G., & Ezer, N. (2004). Halk arasında hemoroet tedavisinde kullanılan bitkiler. Hacettepe University Journal of the Faculty of Pharmacy, (1), 37-60.

Jan, K. N., Zarafshan, K., & Sengh, S. (2017). Stinging nettle (*Urtica dioica* L.): a reservoir of nutrition and bioactive components with great functional potential. Journal of Food Measurement and Characterization, 11, 423-433.

Joshi, B. C., Mukhija, M., & Kalia, A. N. (2014). Pharmacognostical review of *Urtica dioica* L. International Journal of Green Pharmacy (IJGP), 8(4).

Karakaş, S. (2003). Isırgan otu toprak altı ve toprak üstü kısımlarından ısırgan otu ekstraktının eldesi ve özelliklerinin incelenmesi. Doktora Tezi. İstanbul Teknik Üniversitesi, Fen Bilimleri Enstitüsü, Kimya Mühendisliği Ana Bilim Dalı, İstanbul. 142979.

Kavalalı, G. (2011). Materia Medica'dan günümüze uzanan tıbbi bitki: Isırgan. Mersin Üniversitesi Tıp Fakültesi Lokman Hekim Tıp Tarihi ve Folklorik Tıp Dergisi, 1(2), 35-37.

Otles, S. & Yalçın, B. (2012). Phenolic compounds analysis of root, stalk, and leaves of nettle. The Scientific World Journal, 2012. doi:10.1100/2012/564367.

Öztürk, A. İ., & Özdemir, Z. Ö. (2024). Isırgan otu biyoaktif bileşenleri ve analitik yöntemlerle incelenmesi. Bütünleyici ve Anadolu Tıbbı Dergisi, 5(1), 40-50.

Şimşek, I., Aytiken, F., Yesilada, E. & Yıldırımlı, S., 2004. An ethnobotanical study of the Beypazarı, Ayas, and Gudul district towns of Ankara province (Turkey). Economic Botany, 58: 705-720.

Woodland D.W., 1982. Biosystematics of the perennial North American taxa of Urtica. II. Taxonomy. SystematicBotany, 7: 282-290.

Yalçın, B. (2011). Isırgan otundaki (*Urtica dioica*) bazı fenolik bileşiklerin incelenmesi, İzmir.

Yiğen, Ç. (2023). Ülkemizde farklı lokasyonlardan toplanarak Samsun'da yetiştirilen ısırgan otu türlerinin (*Urtica urens* L., *Urtica pilulifera* L.) morfolojik ve kimyasal özelliklerinin belirlenmesi. Doktora Tezi. Ondokuz Mayıs Üniversitesi, Lisansüstü Eğitim Enstitüsü, Tarla Bitkileri Ana Bilim Dalı, Endüstri Bitkileri, Samsun, 831671.

EFFECT OF GERMINATION ON PHYSICOCHEMICAL, CHEMICAL AND NUTRITIONAL PROPERTIES OF LEGUMES

Özge ÖZMEN[1], Osman KOLA[1], Murat Reis AKKAYA[1]

[1] Adana Alparslan Türkeş Science and Technology University, Faculty of Engineering, Department of Food Engineering, Adana-Türkiye

Corresponding author e-mail: ozgeozmen15@gmail.com

ABSTRACT

Legumes are a very healthy and important part of millions of people's diets around the world. All over the world, the production of legumes has grown. The most common types of legumes eaten are beans, lentils, peas, chickpeas, and broad beans. This is because they are low in lipids and high in complex carbohydrates, vitamins, and minerals. Often, their high protein content necessitates their addition to other protein sources. Most of the proteins in legumes are storage proteins. The other proteins are enzymes, as well as two types of macromolecules called antinutrients (lectins and protease inhibitors). Many people germinate legume seeds to make sprouts that you can eat. This simple bioprocess allows legume seeds to have more health benefits. Germination generally enhances the bioavailability of minerals, improves the digestibility of proteins and essential amino acids, and increases the availability of vitamins. It also makes starch, lipids, and antinutritional factors less present in legumes. This process also alters the physicochemical properties of flours derived from germinated legumes, thereby influencing the functional properties of the resulting foods. This study examined the physicochemical, chemical, and nutritional values of legumes following their germination.

Keywords: Legumes, Germination, Bioactive Compounds, Physicochemical Properties, Nutritional Profile

1. INTRODUCTION

Legumes are the third in the classification of chicotyledonous flowering plants, with 750 genera and 16,000-19,000 species. It is part of the *Fabaceas* or *Leguminosae* family (Borges et al., 2013; Sharif et al., 2018). Soybeans, beans, peas, lentils, chickpeas, broad bean, lupine, and peanut are the most well-known legumes in the world (Clemente & Olias, 2017).

Legumes are the second most important food source consumed worldwide after grains due to their nutritional properties and healthiness. Legumes are a high source of protein (20-45%) and are rich in the essential amino acid lysine (Phillips, 1993). It has approximately twice the protein content of grains (Kouris-Blazos & Belski, 2016) and most plant foods in addition to these features, they are also rich in complex carbohydrates (30-60%) and dietary fiber (5-37%) (Kouris-Blazos & Belski, 2016; Phillips, 1993). There are various processes for its consumption, such as soaking, cooking, or germination. Germination is a traditional technique for food processing and is well suited to increase the nutritional value of legumes (Herrera, J., Alizaga, R., Guevara, E., 2006). During germination, seeds (e.g. chickpeas, green lentils) change from a dormant state to a metabolically active state. (Hedley, 2000) This process is affected by biochemical changes that occur depending on germination conditions such as temperature, moisture, time, light, and soaking. During legume germination, bioactive compounds such as essential amino acids, riboflavin, thiamine, biotin, pantothenic acid, niacin, vitamin C, tocopherols, and phenolic compounds increase; It also increases protein digestibility and amino acid availability (Donkor et al., 2012; Khatoon & Prakash, 2006). Germination enhancement also increases reducing sugars, soluble fibers, peptides, and amino acids as well as insoluble phenolic compounds (Hung et al., 2012; Koehler et al., 2007). On the other hand, germination also helps reduce the concentration of antinutritional factors such as alkaloids, trypsin inhibitors, lectins, tannins, and saponins (El-Adawy, 2002). With all these features, the germination process is the most effective and simpler method for improving the nutritional value of legumes due to its ease of use.

1.1. Legumes: A General and Nutritional View

Legumes belong to the *Fabaceae* or *Leguminosae* family, which is the third biggest family among dicotyledonous flowering plants. This family comprises 750 genera and 16,000-19,000 species (Borges et al., 2013; Sharif et al., 2018). They are distributed worldwide due to their ability to adapt to various biotic regions on the planet, such as savannas, humid and dry forests, Mediterranean areas, and temperate regions. This adaptability has led to a wide range of physical characteristics and a diverse array of intrinsic traits (such as nitrogen fixation and reproductive diversity) and extrinsic systems that have played a crucial role in their successful evolution (Koenen et al., 2013). The legumes that are currently the most widely recognized and extensively consumed worldwide are soybeans (*Glycine max*), beans (*Phaseolus vulgaris*), pea (*Pisum sativum*), lentils (*Lens culinaris*), chickpea (*Cicer arietinum*), broad bean (*Vicia faba*), lupine (*Lupinus angustifolius*), and peanut (*Arachis hypogaea*) (Clemente & Olias, 2017).

Legumes have a distinctively low fat content and are abundant in protein and complex carbohydrates, which include dietary fibers. They also have a low glycemic index, as indicated by studies (Duranti, 2006; Gueguen & Cerletti, 1994). Legumes are a valuable source of high quality protein, often containing 20-45% protein content, and are particularly abundant in the important amino acid lysine (Phillips, 1993). Legumes include a greater amount of protein compared to most other plant-based diets, with almost twice the protein content of cereals (Kouris-Blazos & Belski, 2016). Protein legumes and cereals complement each other because grains have significant levels of sulphur amino acids, which are lacking in legumes, while legumes are rich in lysine, which is scarce in cereals (Staniak et al., 2014). Consuming legumes in combination with cereals greatly enhances the protein quality of legumes (Phillips, 1993).

Carbohydrates, which compose 30-60% of legumes, are the second most significant component in terms of nutrition and chemistry. The digestion of starch from legumes is slower compared to starches from tubers and grains. Legumes possess a low glycaemic index (GI) rating, making them appropriate for individuals at a heightened risk of developing diabetes and those who already have the condition (Elhardallou et al., 2013; Phillips, 1993). Legumes are a rich source of dietary fibre, with a content ranging from 5-37%. They include substantial levels of both soluble and insoluble dietary fibre (Kouris-Blazos & Belski, 2016; Phillips, 1993).

In addition, they contain significant quantities of vitamin E, as well as water-soluble vitamins from the B group, including thiamine, niacin, pyridoxine, pantothenic acid, and notably, folate. Furthermore, legumes contain substantial quantities of minerals like calcium, phosphorus, copper, magnesium, iron, zinc, selenium, and potassium, with potassium being the most prevalent among them (Caballero et al., 2015). Essential minerals have crucial physiological functions, including promoting bone health (Ca, P), regulating enzyme activity and iron metabolism (Cu), supporting carbohydrate and lipid metabolism (Cr, Zn), facilitating haemoglobin formation (Fe), and contributing to antioxidative activity, protein synthesis, and plasma membrane stabilization (Zn) (O et al., 2015). Several investigations have shown that the majority of the biologically active substances found in legumes have antioxidant capabilities. These substances are important in preventing chronic degenerative diseases such as hypertension, diabetes, heart disorders, osteoporosis, malignancies, and others (Ndidi et al., 2014).

Legumes include many antinutritional chemicals derived from proteins, such as enzyme inhibitors and lectins, as well as nonprotein components such tannins, phenolics, alkaloids, and phytates (Becerra-Tomás et al., 2018).They disrupt the capacity of certain minerals to be absorbed by the body and hinder the digestion of proteins, leading to negative physiological effects (Aguayo-Rojas et al., 2012) .

Additionally, legumes are considered to be functional foods due to the fact that they are nutrient-dense and offer components that contribute to the enhancement of health. This is because legumes are sources of complex carbohydrates, protein, dietary fiber, and folate, and they also include considerable quantities of vitamins and minerals (Cappa et al., 2018). Nevertheless, legumes are conventionally ingested via a series of procedures including soaking, boiling, and germination. Food processing can alter the nutritional composition of foods, leading to enhancements in their nutritional characteristics (López-Martínez et al., 2017).

1.2. Strategies for the Process of Germination

Germination is a widely used and effective method for enhancing the nutritional properties of seeds. The germination period and presence or absence of light are extrinsic elements that affect this process. These factors either accelerate or hinder the biochemical processes of seed reserve materials (Herrera, J., Alizaga, R., Guevara, E., 2006). In order for germination to occur, several favourable environmental conditions must be present: a moist substrate, sufficient oxygen for aerobic respiration, and an appropriate temperature for metabolic processes and seedling development (López-Amorós et al., 2006).

Germination initiates when the desiccated seed starts to absorb water, triggering a sequence of physiological processes involving cell membranes and metabolic activities in stage I. In stage II, germination concludes as the radicle emerges through the endosperm and the protective coat that enveloped it (Montemurro et al., 2019; Weitbrecht et al., 2011). Various seeds are germinated for human consumption, and the specific germination methods employed depend on the type of seed. These methods typically involve steps such as sterilizing, soaking, and sprouting.

1.2.1. Sterilization

Sterilization is conducted prior to seed soaking to prevent microbial proliferation; however, it is not mandatory for seed germination. The utilization of it will mostly rely on the state of the seeds and the objective of germination. The most frequently utilized sterilization agent for seed germination is a solution containing 0.07% NaClO (María Landete et al., 2015; Pająk et al., 2014). Additionally, ethanol or 70% ethanol has been reported to effectively sterilize seeds within a maximum sterilization period of 3 min (Embaby, 2010; Hooda & Jood, 2003). However, the majority of studies do not perform the sterilization process prior to soaking the seeds. This is likely due to concerns about the possible harmful effects of sterilizing chemicals on the seeds, as well as the potential dangers to food safety for consumers (Gan et al., 2017).

1.2.2. Soaking

Soak seeds in water to make them more water-soluble before they grow. Seeds should be soaked at different times and temperatures, and the weight ratio of the seeds should be followed. Between 24 and 30ºC, seeds can be soaked for 30 minutes to 24 hours at a time, with a water-to-seed ratio of 1:1 to 1:20 (w/v) (Gan et al., 2017; Ghanem et al., 2017; María Landete et al., 2015; Wang et al., 2005). Different conditions for soaking seeds should be linked to things about the seeds, like how thick their coats are, how big they are, and how much water they can hold.

1.2.3. Sprouting

In order to complete this stage of the process, it is essential to place the seeds in incubators or specialized germinators, taking into account parameters such as light, temperature, irrigation, humidity, and time (López-Martínez et al., 2017; Tuan et al., 2019). During the process of sprouting, numerous chemical transformations take place to convert stored carbs and proteins into the developing sprout. This results in the formation of simple carbohydrates, free amino acids, and necessary nutrients that are easily utilized by the body (Duenas et al., 2016). Hence, the process of sprouting greatly enhances the nutritional value of seed sprouts and reduces the presence of antinutrients like α-galactosidases, trypsin inhibitors, and phytic acid (Çavuşoğlu & Kabar, 2010) in the seeds. This makes legume sprouts easier to digest, less likely to cause flatulence, and improves their overall nutritional quality.

1.3. The Impact of The Germination Process on The Physicochemical Characteristics of Legumes.

Germination is an inexpensive method that is highly beneficial for enhancing the nutritional content of leguminous seeds. It improves the digestibility of proteins and carbohydrates, and also increases the concentration of certain vitamins such as riboflavin, niacin, and ascorbic acid. Additionally, germination helps eliminate or reduce the presence of non-nutritional substances found in the seed's reserve compounds (Vidal-Valverde et al., 2002).

Effective methods have been employed to enhance the nutritional and nutraceutical properties of legumes. This involves breaking down and breaking apart the primary nutrients, namely carbohydrates, proteins, and fatty acids. As a result, simple sugars, free amino acids, and organic acids are produced, while the digestibility of starch is increased. Additionally, the hydrolysis of oligosaccharides (raffinose and stachyose) takes place, and the presence of antinutritional and indigestible factors, such as protease inhibitors and lectins, is reduced (Caballero et al., 2015; Monteros et al., 2011; González-Montoya et al., 2018; Saleh et al., 2019). During germination, the levels of reducing sugars, soluble fibre, peptides, amino acids, and non-soluble phenolic compounds increase. However, the specific biochemical changes that occur during germination are influenced by factors such as temperature, humidity, time, light, and soaking (Hung et al., 2012; Koehler et al., 2007). In the process of germination, there is an increase in the quantity of phytase, an enzyme that is responsible for the decomposition of phytic acid (Reddy et al., 1982).

Additionally, germination aids in the reduction of non-nutritional substances such as trypsin inhibitors, lectins, tannins, and saponins. However, it is particularly effective in lowering chemicals like phytic acid, stachyose, and raffinose (El-Adawy, 2002). Germination enhanced the protein characteristics, resulting in a reduction in the surface tension at the air-water interface, facilitating the formation of foam (Kaur et al., 2015). The amphiphilic quality of the protein affects how the flour forms emulsions and how stable it is. The biomolecules around the oil drops changed the type of emulsion that was made. How stable an emulsion is depends on how well the proteins and lipids connect with each other. The stronger the interaction, the more stable the emulsion will be. The better quality of protein and fat in germinated legumes is what makes their emulsification traits better (Sibian et al., 2017). In addition, the process of germination enhances the ability to digest proteins, resulting in changes to the sequence of amino acids. This can result in the creation of peptides that have increased functionality and/or different biological activities compared to those found in ungerminated seeds (Souza Rocha et al., 2015; Saleh et al., 2019).

Sangronis & Machado (2007) found that germinated black beans (*Phaseolus vulgaris L.*), white beans (*Phaseolus vulgaris L.*), and pigeon beans (*Cajanus cajan L. Millsp*) showed a significant reduction in TIA, phytic acid, and tannins, as well as an increase in thiamine, protein digestibility, and ascorbic acid, when compared to their ungerminated seeds. In addition, it was discovered that the process of germination led to a considerable increase in the concentration of ascorbic acid by 300%, 208.4%, and 33.2% in white beans, pigeon beans, and black beans, respectively.

Ghumman, Kuar and Singh (2016) found that the process of germination improved the amount of protein, the ability to foam, the ability to absorb water, and the breakdown viscosity of lentil (Lens culinary) seeds. On the other hand, their stability in foaming, emulsifying, and gravity viscosities decreased. The found rise in protein content might be connected with the production of proteolytic enzymes, which break down proteins into amino acids, break down several compounds, and break down large molecules into simple peptides (Khalil et al., 1995; Uwaegbute et al., 2000).

Alonso et al. (2000), the process of germination enhanced the in vitro protein digestibility (IVPD) of common bean by 10.3%. The researchers determined that this increase could be related to the partial or complete elimination of antinutrients.

Uppal & Bains (2012) showed that germination led to an elevation in vitro protein digestibility in mung bean, chickpea, and cowpea. The rise ranged from 15% to 25% in mung bean and from 6% to 17% in both chickpea and cowpea, as compared to the seeds that were not germinated. In addition, they discovered that the in vitro starch digestibility had a considerable rise ($p<0.05$) following germination. The documented percentage rise ranged from 8 to 12% in mung bean, 9 to 11% in chickpea, and 10 to 13% in cowpea, in comparison to their respective unfermented seeds.

Sangronis & Machado (2007) found that the digestibility of black and white bean proteins rose by 3% following a 5 day germination period of the seeds.

Mubarak (2005) found that the levels of phytic acid and tannins in mung bean (Phaseolus aureus) seeds were dramatically decreased by the processes of germination and cooking, with a statistical significance of $p<0.05$. Both methods also greatly enhanced the in vitro protein digestibility of mung bean seeds. The enhanced digestibility can be related to the denaturation of protein, deactivation of trypsin inhibitors, and reduction of tannins and phytic acid levels resulting from the processes of germination and cooking.

Gaafar et al. (2017) found that extrusion heating of germinated chickpea seeds (*Cicer arietinum L.*) resulted in a notable reduction (p<0.05%) in the levels of protein and fat in the extrudes. However, the levels of fibre, ash, and carbs were not impacted. Additionally, they observed a substantial reduction in the levels of phytic and tannic acid, ranging from 40.64% to 46.07% and 40.46% to 44.88%, respectively.

Dikshit & Ghadle (2003)reported that the process of germination led to a significant enhancement in the digestibility of soy proteins, with an increase of up to 39%. Alonso et al. (2000)observed a rise in protein digestibility. Previous research has demonstrated that the presence of bean trypsin inhibitors decreases by 27% and 84% after a period of 2-3 days of germination, as shown in related studies (Alonso et al., 1998; Bishnoi & Khetarpaul, 1994; Hobday et al., 1973). Conversely, the red bean experiences a decrease in antitrypsin levels ranging from 17% to 63% following intervals of 1 to 10 days (Alonso et al., 2000; El-Hag et al., 1978; Sathe et al., 1983). Studies have shown that germination of lentils can lead to reductions in the range of 21 to 54% after 4 and 6 days, respectively (El-Mahdy et al., 1985). According to the studies conducted by Vidal-Valverde et al. (2002) and Frias et al. (1997), inhibitors are utilized as a source of energy for the growth and development of seedlings. In contrast, the reduction percentages in chickpea germinated for 3-6 days range from 24% to 83% (Bansal et al., 1988). According to Wilson (1980)the decrease in legumes may be caused by the enzymatic mobilization and breakdown of proteins, such as trypsin inhibitors, during the germination of the seeds. Nevertheless, Ryan (1983)'s research indicate that this decrease is not associated with any specific biochemical mechanism during seed germination.

Rusydi et al. (2012) conducted an analysis on the phytic acid content in germinated soybeans (at a temperature of 28°C for 48 hours) and non-germinated soybeans. They observed a reduction in phytic acid concentration from 63.35 to 49.58 mg/100g following germination. The decrease in phytate content could be attributed to the activation of the naturally occurring phytase enzyme during the germination process.

Crans et al. (1995) discovered that after 6 days of germination, beans and peas saw a 60% reduction in phytic acid. The varying reduction percentages observed in common bean and other legumes are likely attributed to the distinct methods by which phytic acid is broken down in each species, namely the differential activity of endogenous phytases. The reduction of phytic acid through hydrolysis increases the nutritional value of the seed. This process releases phosphorus that can be absorbed during digestion. Additionally, it enhances the bioavailability of minerals that are naturally linked to this compound as salts.

Jiang et al. (2013) observed a 50% rise in the overall phenolic content and a decrease of 27.5% and 35% in the levels of phytic acid and trypsin inhibitory activity, respectively, following a 50 hour germination period. A study has been conducted to examine the impact of germination on the composition and distribution of isoflavones in soymilk.

CONCLUSIONS

With the growing understanding of the importance of legumes around the world, their importance in human nutrition has increased. There are various methods of consuming legumes. One of these is the germination of legumes. Studies show that germinated legumes make minerals more accessible, proteins and important amino acids more digestible, and vitamins more available. It also makes starch, lipids, and antinutritional factors less present in legumes. Many studies show that the amount of phytic acid, an anti-nutritional factor, decreases with the germination of legumes. As a result, the germination of legumes is a cheap method and has many nutritional benefits.

REFERENCES

Aguayo-Rojas, J., Mora-Rochín, S., Cuevas-Rodríguez, E. O., Serna-Saldivar, S. O., Gutierrez-Uribe, J. A., Reyes-Moreno, C., & Milán-Carrillo, J. (2012). Phytochemicals and Antioxidant Capacity of Tortillas Obtained after Lime-Cooking Extrusion Process of Whole Pigmented Mexican Maize. *Plant Foods for Human Nutrition*, *67*(2). https://doi.org/10.1007/s11130-012-0288-y

Alonso, R., Aguirre, A., & Marzo, F. (2000). Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. *Food Chemistry*, *68*(2). https://doi.org/10.1016/S0308-8146(99)00169-7

Alonso, R., Orúe, E., & Marzo, F. (1998). Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Food Chemistry*, *63*(4). https://doi.org/10.1016/S0308-8146(98)00037-5

Bansal, K. (1988). Trypsin inhibitor and hemagglutinin activities in chickpea (Cicer arietinum L.): effects of heat and germination. *Journal of Food Science*.

Becerra-Tomás, N., Díaz-López, A., Rosique-Esteban, N., Ros, E., Buil-Cosiales, P., Corella, D., Estruch, R., Fitó, M., Serra-Majem, L., Arós, F., Lamuela-Raventós, R. M., Fiol, M., Santos-Lozano, J. M., Díez-Espino, J., Portoles, O., Salas-Salvadó, J., Serra-Mir, M., Pérez-Heras, A., Viñas, C., … Timiraus-Fernández, J. (2018). Legume consumption is inversely associated with type 2 diabetes incidence in adults: A prospective assessment from the PREDIMED study. *Clinical Nutrition*, *37*(3). https://doi.org/10.1016/j.clnu.2017.03.015

Bishnoi, S., & Khetarpaul, N. (1994). Protein digestability of vegetables and field peas (Pisum sativum) - Varietal differences and effect of domestic processing and cooking methods. *Plant Foods for Human Nutrition*, *46*(1). https://doi.org/10.1007/BF01088463

Borges, L., Bruneau, A., Cardoso, D., Crisp, M., Delgado-Salinas, A., Doyle, J. J., Egan, A., Herendeen, P. S., Hughes, C., Kenicer, G., Klitgaard, B., Koenen, E., Lavin, M., Lewis, G., Luckow, M., Mackinder, B., Malécot, V., Miller, J. T., Pennington, R. T., … Wink, M. (2013). Towards a new classification system for legumes: Progress report from the 6th International Legume Conference. *South African Journal of Botany*, *89*, 3–9. https://doi.org/10.1016/j.sajb.2013.07.022

Caballero, B., Finglas, P., & Toldrá, F. (2015). *Encyclopedia of food and health*.

Cappa, C., Kelly, J. D., & Ng, P. K. W. (2018). Seed characteristics and physicochemical properties of powders of 25 edible dry bean varieties. *Food Chemistry*, *253*. https://doi.org/10.1016/j.foodchem.2018.01.048

Çavuşoğlu, K., & Kabar, K. (2010). Effects of hydrogen peroxide on the germination and early seedling growth of barley under NaCl and high temperature stresses. *EurAsian Journal of Biosciences*. https://doi.org/10.5053/ejobios.2010.4.0.9

Clemente, A., & Olias, R. (2017). Beneficial effects of legumes in gut health. *Current Opinion in Food Science*, *14*, 32–36. https://doi.org/10.1016/j.cofs.2017.01.005

Crans, D. C., Mikuš, M., & Blayne Friehauf, R. (1995). Phytate Metabolism in Bean Seedlings duringPost-Germinative Growth. *Journal of Plant Physiology*, *145*(1–2). https://doi.org/10.1016/S0176-1617(11)81854-7

de los Monteros, L. A. G. E., Ramón-Gallegos, E., Torres-Torres, N., & Mora-Escobedo, R. (2011). Effect of Germinated Soybean Protein Hydrolysates on Adipogenesis and Adipolysis in 3T3-L1 Cells. *Plant Foods for Human Nutrition*, *66*(4). https://doi.org/10.1007/s11130-011-0263-z

de Souza Rocha, T., Hernandez, L. M. R., Mojica, L., Johnson, M. H., Chang, Y. K., & González de Mejía, E. (2015). Germination of Phaseolus vulgaris and alcalase hydrolysis of its proteins produced bioactive peptides capable of improving markers related to type-2 diabetes in vitro. *Food Research International*, *76*(P1). https://doi.org/10.1016/j.foodres.2015.04.041

Dikshit, M., & Ghadle, M. (2003). Effect of sprouting on nutrients, antinutrients and in vitro digestibility of the MACS-13 soybean variety. *Plant Foods for Human Nutrition*, *58*(3), 1–11. https://doi.org/10.1023/B:QUAL.0000040357.70606.4C

Donkor, O. N., Stojanovska, L., Ginn, P., Ashton, J., & Vasiljevic, T. (2012). Germinated grains – Sources of bioactive compounds. *Food Chemistry*, *135*(3), 950–959. https://doi.org/10.1016/j.foodchem.2012.05.058

Duenas, M., Sarmento, T., & Aguilera, Y. (2016). Impact of cooking and germination on phenolic composition and dietary fibre fractions in dark beans (Phaseolus vulgaris L.) and lentils (Lens culinaris L.). *Food Science and Technology*.

Duranti, M. (2006). Grain legume proteins and nutraceutical properties. In *Fitoterapia* (Vol. 77, Issue 2). https://doi.org/10.1016/j.fitote.2005.11.008

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition*, *57*(1), 83–97. https://doi.org/10.1023/A:1013189620528

El-Hag, N., Haard, N. F., & Morse, R. E. (1978). Influence of sprouting on the digestibility coefficient, trypsin inhibitor and globulin proteins of red kidney beans. *Journal of Food Science*, *43*(6), 1874–1875. https://doi.org/10.1111/J.1365-2621.1978.TB07435.X

Elhardallou, S. B., Khalid, I. I., & Balla Elharadallou, S. (2013). Functional properties of cowpea (Vigna ungiculata L. Walp), and lupin (Lupinus termis) flour and protein isolates. *Journal of Nutrition & Food Sciences*, *3*(6). https://doi.org/10.4172/2155-9600.1000234

El-Mahdy, A. R., Moharram, Y. G., & Abou-Samaha, O. R. (1985). Influence of germination on the nutritional quality of lentil seeds. *Zeitschrift Für Lebensmittel-Untersuchung Und -Forschung*, *181*(4). https://doi.org/10.1007/BF01043094

Embaby, H. E. S. (2010). Effect of soaking, dehulling, and cooking methods on certain antinutrients and in vitro protein digestibility of bitter and sweet lupin seeds. *Food Science and Biotechnology*, *19*(4). https://doi.org/10.1007/s10068-010-0148-1

Frias, J., Kovács, E., Sotomayor, C., Hedley, C., & Vidal-Valverde, C. (1997). Processing peas for producing macaroni. *European Food Research and Technology*, *204*(1). https://doi.org/10.1007/s002170050039

Gaafar, A., Hegazy, H. S., El-Fath, A., El-Bedawey, A., Rahma, E.-S. H., & Gaafar, A. M. (2017). Effect of extrusion process on nutritional, functional properties and antioxidant activity of germinated chickpea incorporated corn extrudates. *Food Sci. Nutr.* , *4*(1), 59–66.

Gan, R. Y., Lui, W. Y., Wu, K., Chan, C. L., Dai, S. H., Sui, Z. Q., & Corke, H. (2017). Bioactive compounds and bioactivities of germinated edible seeds and sprouts: An updated review. In *Trends in Food Science and Technology* (Vol. 59). https://doi.org/10.1016/j.tifs.2016.11.010

Ghanem, M. E., Kibbou, F. ez zahra, Guiguitant, J., & Sinclair, T. R. (2017). Opportunities to improve the seasonal dynamics of water use in lentil (Lens culinaris Medik.) to enhance yield increase in water-limited environments. In *Chemical and Biological Technologies in Agriculture* (Vol. 4, Issue 1). https://doi.org/10.1186/s40538-017-0103-y

Ghumman, A., & Kaur, A. (2016). Impact of germination on flour, protein and starch characteristics of lentil (Lens culinari) and horsegram (Macrotyloma uniflorum L.) lines. *LWT-Food Science and Technology*. https://www.sciencedirect.com/science/article/pii/S002364381530092X

González-Montoya, M., Hernández-Ledesma, B., Silván, J. M., Mora-Escobedo, R., & Martínez-Villaluenga, C. (2018). Peptides derived from in vitro gastrointestinal digestion of germinated soybean proteins inhibit human colon cancer cells proliferation and inflammation. *Food Chemistry*, *242*. https://doi.org/10.1016/j.foodchem.2017.09.035

Gueguen, J., & Cerletti, P. (1994). Proteins of some legume seeds: soybean, pea, fababean and lupin. In *New and Developing Sources of Food Proteins*. https://doi.org/10.1007/978-1-4615-2652-0_6

Herrera J, Alizaga R, & Guevara E. (2006). *Germinación y crecimiento de la planta. Fisiología de la producción de los cultivos tropicales.*

Hobday, S. M., Thurman, D. A., & Barber, D. J. (1973). Proteolytic and trypsin inhibitory activities in extracts of germinating Pisum sativum seeds. *Phytochemistry*, *12*(5). https://doi.org/10.1016/0031-9422(73)85012-5

Hooda, S., & Jood, S. (2003). Effect of soaking and germination on nutrient and antinutrient contents of fenugreek (Trigonella foenum graecum L.). *Journal of Food Biochemistry*, *27*(2). https://doi.org/10.1111/j.1745-4514.2003.tb00274.x

Hung, P. Van, Maeda, T., Yamamoto, S., & Morita, N. (2012). Effects of germination on nutritional composition of waxy wheat. *Journal of the Science of Food and Agriculture*, *92*(3), 667–672. https://doi.org/10.1002/jsfa.4628

Jiang, S., Cai, W., & Xu, B. (2013). *Food Quality Improvement of Soy Milk Made from Short-Time Germinated Soybeans*. *2*, 198–212. https://doi.org/10.3390/foods2020198

Kaur, M., Sandhu, K. S., Ahlawat, R. P., & Sharma, S. (2015). In vitro starch digestibility, pasting and textural properties of mung bean: effect of different processing methods. *Journal of Food Science and Technology*, *52*(3). https://doi.org/10.1007/s13197-013-1136-2

Khalil, A., & Mansour E. (1995). The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. *Food Chemistry*.

Khatoon, N., & Prakash, J. (2006). Nutrient retention in microwave cooked germinated legumes. *Food Chemistry*, *97*(1), 115–121. https://doi.org/10.1016/j.foodchem.2005.03.007

Koehler, P., Hartmann, G., Wieser, H., & Rychlik, M. (2007). Changes of Folates, Dietary Fiber, and Proteins in Wheat As Affected by Germination. *Journal of Agricultural and Food Chemistry*, *55*(12), 4678–4683. https://doi.org/10.1021/jf0633037

Koenen, E. J. M., de Vos, J. M., Atchison, G. W., Simon, M. F., Schrire, B. D., de Souza, E. R., de Queiroz, L. P., & Hughes, C. E. (2013). Exploring the tempo of species diversification in legumes. *South African Journal of Botany*, *89*, 19–30. https://doi.org/10.1016/j.sajb.2013.07.005

Kouris-Blazos, A., & Belski, R. (2016). Health benefits of legumes and pulses with a focus on Australian sweet lupins. In *Asia Pacific Journal of Clinical Nutrition* (Vol. 25, Issue 1). https://doi.org/10.6133/apjcn.2016.25.1.23

López-Amorós, M. L., Hernández, T., & Estrella, I. (2006). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition and Analysis*, *19*(4), 277–283. https://doi.org/10.1016/j.jfca.2004.06.012

López-Martínez, L. X., Leyva-López, N., Gutiérrez-Grijalva, E. P., & Heredia, J. B. (2017). Effect of cooking and germination on bioactive compounds in pulses and their health benefits. *Journal of Functional Foods*, *38*, 624–634. https://doi.org/10.1016/j.jff.2017.03.002

María Landete, J., Hernández, T., Robredo, S., Dueñas, M., De Las Rivas, B., Estrella, I., & Muñoz, R. (2015). Effect of soaking and fermentation on content of phenolic compounds of soybean (Glycine max cv. Merit) and mung beans (Vigna radiata [L] Wilczek). *International Journal of Food Sciences and Nutrition*, *66*(2). https://doi.org/10.3109/09637486.2014.986068

Mubarak, A. E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes. *Food Chemistry*, *89*(4). https://doi.org/10.1016/j.foodchem.2004.01.007

Ndidi, U. S., Ndidi, C. U., Aimola, I. A., Bassa, O. Y., Mankilik, M., & Adamu, Z. (2014). Effects of Processing (Boiling and Roasting) on the Nutritional and Antinutritional Properties of Bambara Groundnuts (Vigna subterranea [L.] Verdc.) from Southern Kaduna, Nigeria. *Journal of Food Processing*. https://doi.org/10.1155/2014/472129

O, M., K, M., & W, R. L. M. (2015). Essential mineral content of common fish species in Chanoga, Okavango Delta, Botswana. *African Journal of Food Science*, *9*(9). https://doi.org/10.5897/ajfs2015.1307

Pająk, P., Socha, R., Gałkowska, D., Rożnowski, J., & Fortuna, T. (2014). Phenolic profile and antioxidant activity in selected seeds and sprouts. *Food Chemistry*, *143*. https://doi.org/10.1016/j.foodchem.2013.07.064

Phillips, R. D. (1993). Starchy legumes in human nutrition, health and culture. *Plant Foods for Human Nutrition*, *44*(3). https://doi.org/10.1007/BF01088314

Reddy, N. R., Sathe, S. K., & Salunkhe, D. K. (1982). *Phytates in Legumes and Cereals* (Vol. 28, pp. 1–92). https://doi.org/10.1016/S0065-2628(08)60110-X

Rusydi, M., & Megat A. (2012). Effect of germination on total phenolic, tannin and phytic acid contents in soy bean and peanut. *International Food Research Journal*.

Ryan, C. A. (1983). Wound-Regulated Synthesis and Compartmentation of Proteinase Inhibitors in Plant Leaves. *Structure and Function of Plant Genomes*, 337–346. https://doi.org/10.1007/978-1-4684-4538-1_33

Saleh, H. M., Hassan, A. A., Mansour, E. H., Fahmy, H. A., & El-Bedawey, A. E. F. A. (2019). Melatonin, phenolics content and antioxidant activity of germinated selected legumes and their fractions. *Journal of the Saudi Society of Agricultural Sciences*, *18*(3). https://doi.org/10.1016/j.jssas.2017.09.001

Sangronis, E., & Machado, C. J. (2007). Influence of germination on the nutritional quality of Phaseolus vulgaris and Cajanus cajan. *LWT - Food Science and Technology*, *40*(1), 116–120. https://doi.org/10.1016/j.lwt.2005.08.003

Sathe, S. K., Deshpande, S. S., Reddy, N. R., Goll, D. E., & Salundake, D. K. (1983). Effects of Germination on Proteins, Raffinose Oligosaccharides, and Antinutritional Factors in the Great Northern Beans (Phaseolus vulgaris L.). *Journal of Food Science*, *48*(6). https://doi.org/10.1111/j.1365-2621.1983.tb05087.x

Sharif, H. R., Williams, P. A., Sharif, M. K., Abbas, S., Majeed, H., Masamba, K. G., Safdar, W., & Zhong, F. (2018). Current progress in the utilization of native and modified legume proteins as emulsifiers and encapsulants – A review. *Food Hydrocolloids*, *76*, 2–16. https://doi.org/10.1016/j.foodhyd.2017.01.002

Sibian, M. S., Saxena, D. C., & Riar, C. S. (2017). Effect of germination on chemical, functional and nutritional characteristics of wheat, brown rice and triticale: a comparative study. *Journal of the Science of Food and Agriculture*, *97*(13). https://doi.org/10.1002/jsfa.8336

Staniak, M., Ksiak, J., & Bojarszczuk, J. (2014). Mixtures of Legumes with Cereals as a Source of Feed for Animals. In *Organic Agriculture Towards Sustainability*. https://doi.org/10.5772/58358

Tuan, P., Sun, M., Nguyen, T., Park, S., & Ayele BT. (2019). Molecular mechanisms of seed germination. *Sprouted Grains*. https://www.sciencedirect.com/science/article/pii/B9780128115251000014

Uppal, V., & Bains, K. (2012). Effect of germination periods and hydrothermal treatments on in vitro protein and starch digestibility of germinated legumes. *Journal of Food Science and Technology*, *49*(2). https://doi.org/10.1007/s13197-011-0273-8

Uwaegbute, A., Iroegbu, C., & Eke O. (2000). Chemical and sensory evaluation of germinated cowpeas (Vigna unguiculata) and their products. *Food Chemistry*. https://www.sciencedirect.com/science/article/pii/S030881469900134X

Vidal-Valverde, C., Frias, J., Sierra, I., & Blazquez I. (2002). New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *Food Research and Technology*, *215*(6), 472–477. https://doi.org/10.1007/s00217-002-0602-2

Wang, K. H., Lai, Y. H., Chang, J. C., Ko, T. F., Shyu, S. L., & Chiou, R. Y. Y. (2005). Germination of peanut kernels to enhance resveratrol biosynthesis and prepare sprouts as a functional vegetable. *Journal of Agricultural and Food Chemistry*, *53*(2). https://doi.org/10.1021/jf048804b

Wilson KA. (1980). The release of proteinase inhibitors from legume seeds during germination. *Phytochemistry*.

IMPACT OF LEGUME GERMINATION ON BIOACTIVE COMPOUNDS AND THEIR BENEFITS TO HUMAN HEALTH

Özge ÖZMEN[1], Osman KOLA[1], Murat Reis AKKAYA[1]

[1] Adana Alparslan Türkeş Science and Technology University, Faculty of Engineering, Department of Food Engineering, Adana-Türkiye

Corresponding author e-mail: ozgeozmen15@gmail.com

ABSTRACT

Plants that are legumes are in the family *Leguminosae*. Chickpeas, lentils, beans and peas are the varieties of legumes that are consumed the most commonly in the worldwide. They have a high nutritional content and are an excellent source of proteins, dietary fiber, vitamins and minerals. The germination of legumes is a simple and inexpensive process. Recent research has shown that the process of germination can further enhance the medical and nutritional properties of legumes. In addition, germination has the potential to enhance human nutrition and health by preventing malnutrition and chronic diseases. This is because it can augment the levels of certain nutrients, bioactive compounds, and biological activities in edible seeds. Germination can accumulate various bioactive compounds in germinated legumes, such as g-aminobutyric acid (GABA), polyphenols and vitamins. Several biological activities, including as anti-inflammatory, antidiabetic, anticancer, antioxidant, antibacterial, antihypertensive and immunomodulatory capabilities, have been discovered for bioactive compounds as a result of their investigation. Overall, germinated legumes rich in bioactive compounds and nutrients can be considered for the prevention of some chronic diseases and malnutrition as functional foods. In this study, the effect of germination on bioactive components in legumes and their benefits for human health were examined.

Keywords: Bioactive compounds, Legumes, Germination of legumes, Human health

1. INTRODUCTION

Numerous bioactive compounds found in legumes may provide defense against metabolic illnesses (Raju & Mehta, 2008). Because they have so many bioactive ingredients, they are an essential cereal alternative, especially for those watching what they eat. They are therefore a great choice for people who are disease (Maphosa & Jideani, 2017). A popular conventional method that increases nutrient digestibility and boosts bioactive and anti-nutritional components while reducing some of the latter is germination (Rizvi et al., 2022). Depending on the seed type and the circumstances around germination, some changes may take place inside the seed during the soaking and germination process. The most effective method for lowering pulses' anti-nutritional chemicals is reported to be germination (Sangronis & Machado, 2007). Bioactive compounds like pantothenic acid, riboflavin, thiamine, biotin, biotin, essential amino acids, vitamin C, tocopherols, and phenolic compounds increase during

legume germination. It also increases the the accessibility of amino acids and the digestibility of protein (Donkor et al., 2012; Khatoon & Prakash, 2006). Along with insoluble phenolic chemicals, germination improvement also results in an increase in reducing amino acids, peptides, soluble fibers, and sugars (Hung et al., 2012; Koehler et al., 2007). Conversely, germination also aids in lowering the concentration of antinutritional elements such saponins, tannins, lectins, trypsin inhibitors, and alkaloids (El-Adawy, 2002).
Furthermore, because they are rich in nutrients and provide elements that support improved health, legumes are regarded as functional foods. This is due to the fact that legumes contain significant amounts of vitamins and minerals along with complex carbs, protein, dietary fiber, and folate (Cappa et al., 2018).

2. Impact of legume germination on bioactive compounds

Legumes are considered to be a viable economic and nutritionally significant part of the human diet. The variety of their nutritious components, which include complex carbohydrates (60%), dietary fiber (3-57%), and proteins (20-45%), shows to their quality. Furthermore, the fat content of legumes is generally low (~5%), with the exception of chickpeas (15%), and much higher (45-50%) in the case of peanuts and soybeans. Legumes also include a variety of bioactive substances that may offer protection against metabolic diseases (Raju & Mehta, 2008). Owing to their low glycemic index and a lot of bioactive components, they represent a vital substitute for cereals, particularly for individuals who are careful of their nutritional intake. As such, they are an excellent option for individuals with celiac disease and diabetes (Maphosa & Jideani, 2017).

The biological process of germination is one that results in the transformation of the embryo into a plant by enzyme activation in legumes, cereals, and pseudo-cereals. Three different phases can be recognized in germination (Aguilar et al., 2019; Cho & Lim, 2016). The dry grain rapidly absorbs water in the first phase (phase I), which causes hydration and activates intercellular enzymes. This starts the metabolic processes that have been inactive to resume, and then there is a period of restricted water absorption. Important irreversible metabolic processes occur during Phase II, such as the mobilization of resultant small molecules (low molecular weight proteins and peptides, fatty acids, sugars), to various seed parts and the breakdown of reserve molecules by enzymes (high molecular weight proteins, triglycerides and starch). Phase III sees a modest rise in water content, particularly in the radicle cells that are increasing due to mitotic divisions and cell development in the seedlings. Some big seeds that have a significant molecular reserve may continue to absorb water after the radicles emerge. Genetic variations and compositional changes account for the variation in the pattern of water absorption, enzymatic reactions, and size among plant species (Choque-Quispe et al., 2021). According to research (Urbano et al., 1995; Vidal-Valverde et al., 2002, 2003), legumes that have undergone germination tend to have higher levels of dietary fiber, accessible carbohydrates, free amino acids, and other nutrients while having fewer antinutritive chemicals present. It also increases the levels of bioactive compounds like phenolics and flavonoids, which increases nutrient absorption (Miyahira et al., 2021).

Antinutrient content is decreased during the germination phase, whereas antioxidant component content and release-such as those of vitamins, polyphenols and minerals are greatly increased. This could be as a result of germination increasing content by release (e.g., cotyledon cell structure disintegration activates hydrolytic enzymes) or biosynthesis (e.g., de novo production of vitamins or polyphenols) (Alkaltham et al., 2020). Starch digestibility rises as a result of the breakdown of fats and carbohydrates, which are frequently consumed in abundance in western diets (Jyothi & PR, 1981; Subbulakshmi et al., 1976; Vidal-Valverde & Frias, 1992) Both dietary fiber and phytotic acid have an impact on the digestive tract's ability to absorb micronutrients, and they undergo distinct changes throughout the germination process (Pawar et al., 1986; Vidal-Valverde & Frias, 1992). Moreover, a recent study shown that the germination of lentils and fava beans enhances the release of bioactive peptides and amino acids during digestion. The length of time that seeds take to germinate, grow sprouts, and speed up the process of proteolysis are all significantly influenced by their permeability. Antinutrient degradation and seed microstructure disintegration affect protein digestibility and the release of bioactive peptides during digestion (Bautista-Expósito et al., 2021). During germination, the digestibility of protein in chickpea was twice as high as in raw chickpea (Milán-Noris et al., 2018) the amount of flavonoids and polyphenols also increased significantly (Gupta et al., 2017). During germination, the amount of oligosaccharides, α-galactosides that cause flatulence, and chymotrypsin and trypsin inhibitors that influence protein digestion can all be decreased (Frias et al., 1995; Urbano et al., 1995; Vidal-Valverde et al., 1994; Vidal-Valverde & Frias, 1992).

3. Benefits to human health of germinated legumes

3.1. Vitamins and Minerals

Prodanov et al. (1997) reported that the quantity of riboflavin, total accessible niacin and thiamine in faba beans and lentils changed depending on the frequency of washing, the availability of light, and the germination time when seeds were processed in a separating funnel at 20ºC. Vidal-Valverde et al. (2002) observed the germination of beans, peas, and lentils on different days, as well as the levels of total inositol phosphate, total soluble sugar, B1 and B2. As a result of the prolonged germination durations for all three species, the total vitamin B2 and sugar increased while the total inositol phosphate content and vitamin B1 dropped. Abdullah et al. (1984) observed a increase of ascorbic acid, riboflavin, thiamin and niacin after the germination of mung bean and soybean for 3 days. After peas germinated for six days in the dark, vitamin B1 declined by 83% while vitamin B2 became by two times, according to Sierra et al. (1999). The plant species, maturity, environment, harvesting and storage practices, and vitamin content all affect how long a seed takes to germinate and how much of it takes (Masood et al., 2014).

Urbano et al. (2006) observed that in their experiments where Mg and Zn sprouted at different periods in peas, their bioavailability was higher on the second and fourth days.

3.2. Antioxidant activity

Through the elimination of free radicals from cells, antioxidants play a vital part in preventing the deterioration of degenerative diseases and food. To protect cell membrane lipids from polyunsaturated fatty acid (PUFA) peroxidation, fat-soluble antioxidants like carotenoids and tocopherols work against in the aqueous phase, reactive oxygen species (ROS). Hydrophilic antioxidants, on the other hand, counteract ROS in the water phase. Both hydrophobic and hydrophilic phases of phenolic compounds, such as flavonoids, demonstrate antioxidant potential (Munteanu et al., 2021).

Antioxidant component content and availability may increase during germination; this can be attributed to the release of antioxidant chemicals from of the disintegration of cell walls or to the production of bioactive compounds by means of secondary metabolic pathways being activated. Loss of these compounds can sometimes be linked to low molecular weight polyphenolic compound polymerization, complex formation with proteins, or leaching during soaking (Bhinder et al., 2021).

Past research confirms that an increase in germination time correlates with an increase in antioxidant activity; López-Amorós et al. (2006a) also noted this pattern. In a similar vein, studies conducted by Saleh et al. (2019) provided evidence that an increase in germination time was connected to an elevation in antioxidant activity. The active macro and micro components that are already present in the seed are what are responsible for this increase in antioxidant capability.

The defenses that phenolic compounds provide against oxidative stress are crucial.(Gopi et al., 2007; Pedranzani et al., 2007). The content of phenolic compounds in germinated beans was positively connected to an intensify in antioxidant capability. After five days of germination, the phenolic compound content of ungerminated seeds significantly increased in soybeans, beans and peanuts.(Tan Khang et al., 2016).

The TPC (Total Phenolic Compound) of pulses (red lentil, chickpea and kidney bean) varied for non-germinated grains, ranging from 0.6 to 1.1 g/kg, per (Mamilla & Mishra, 2017). Due of the thicker seed coat and enhanced polyphenol oxidase activity, which decreased water absorption, germination changed the TPC of red lentil and chickpea but not kidney beans (Boesewinkel & Bouman, 2018).

Depending on the pulse, the TPC increased during germination at 30ºC and 40ºC, respectively, between 0.8 and 1.82 g/kg and 0.75 to 1.1 g/kg. Lin & Lai (2006) made similar observations. At 40ºC, the antioxidant activity (AA) was at its greatest. With germination, the total flavonoid content (TFC) of the following increased: 0.19 to 0.32 g/kg for kidney beans, 0.17 to 0.25 g/kg for red lentils, and 0.22 to 0.42 g/kg for chickpeas. At 30ºC for chickpea germination, and 40ºC for red lentil and kidney bean germination, TFC rose. These variations might result from variations in how the germination rates depend on temperature.

Legume species belonging to Homogalgena (lentil and chickpea) often have a lower germination temperature (30ºC) than phaseoloid-clad legumes (kidney bean) (Mierziak et al., 2014). The antioxidant activity and phenolic content of lentils that have germinated have been investigated by Gharachorloo et al. (2013) . The total phenolic content of sprouted lentils was found to be higher (78 mg/kg) than that of raw lentil seeds (53 mg/kg) after 5 days of germination.

Cevallos-Casals&Cisneros-Zevallos (2010) measured the concentrations of phenolic compounds in faba seeds at various stages of germination. 7 days sprouts>dormant seeds>ingested seeds was the general trend distribution of accumulated phenolics and total antioxidant capacity. Seeds are an excellent source of phenolic antioxidants for food because the phenols released during germination may lead to greater phenol concentrations and antioxidant activity.

The investigation conducted by López-Amorós et al. (2006b) looked at how germination affected the phenolic profile. The results of the study demonstrated that the process of germination altered the phenolic profile of the legumes, affecting both the flour's functional qualities and antioxidant capacity. According to Sokrab et al. (2012), germination causes the phenolic content of germinated seeds to become soluble, which causes its content to increase. Furthermore, as evidenced by the browning of the germinated seeds, Sokrab et al. (2012) and Duenas et al. (2009) found that germination causes condensed tannins to become soluble and phenolic compounds to move to the outer layer. Consequently, the germinated seeds exhibit an increase in their overall phenolic content. Additionally, during seed germination, the enzyme systems are activated and mobilized, which decreases the concentration of the hydrophilic component of the seed and, therefore, the amounts of tannin, anthocyanin, and total phenolics. (Sokrab et al., 2012).

Chauhan et al. (2022) studied how black soybean nutrients, anti-nutrients, and bioactive components were affected by soaking, germination, fermentation, and roasting. The phenolic contents increased dramatically (p<0.05) throughout germination, roasting and fermentation according to the results. During germination and fermentation, treated grains exhibited a considerable (p<0.05) increase in antioxidant activity. Phytic acid and tannin levels, two anti-nutritional chemicals, dramatically dropped following processing. The primary causes of the surge in sprout intake are convenience and complex physiological modifications that enhance the nutritional profile and reduce absorption-hindering antinutrients.

CONCLUSIONS

The germination process means sprouting legumes (such as chickpeas, lentils, beans) and this process can lead to various positive changes in their nutritional values. The germination process increases the proteins' bioavailability and digestibility, increases the levels of some vitamins and minerals such as vitamin C, vitamin B, folate and antioxidants, and reduces antinutritional factors in legumes. The bioactives that come from legumes undergo major modifications as a result of the germination process.

The health benefits of these bioactives have been demonstrated in various studies. However, the health effects of germination and legume-based products need further in vitro and in vivo research.

REFERENCES

Abdullah, A., Baldwin, R., & Minor, H. (1984). Germination effects on flatus-causing factors and antinutrients of mungbeans and two strains of small-seeded soybeans. *Journal of Food Protection*.

Aguilar, J., Miano, A. C., Obreg, J., Soriano-Colchado, J. E., & Barraza-J Auregui, G. (2019). Malting process as an alternative to obtain high nutritional quality quinoa flour. *Elsevier*. https://doi.org/10.1016/j.jcs.2019.102858

Alkaltham, M., Salamatullah, A., Özcan, M., & Uslu, N. (2020). The effects of germination and heating on bioactive properties, phenolic compounds and mineral contents of green gram seeds. *LWT*.

Bautista-Expósito, S., Vandenberg, A., Peñas, E., Frias, J., & Martínez-Villaluenga, C. (2021). Lentil and Fava Bean With Contrasting Germination Kinetics: A Focus on Digestion of Proteins and Bioactivity of Resistant Peptides. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/FPLS.2021.754287/FULL

Bhinder, S., Kumari, S., Singh, B., Kaur, A., & Singh, N. (2021). Impact of germination on phenolic composition, antioxidant properties, antinutritional factors, mineral content and Maillard reaction products of malted quinoa flour. *Food Chemistry*.

Boesewinkel, F. D., & Bouman, F. (2018). The Seed: Structure and Function. *Seed Development and Germination*, 1–24. https://doi.org/10.1201/9780203740071

Cappa, C., Kelly, J. D., & Ng, P. K. W. (2018). Seed characteristics and physicochemical properties of powders of 25 edible dry bean varieties. *Food Chemistry*, *253*. https://doi.org/10.1016/j.foodchem.2018.01.048

Cevallos-Casals, B. A., & Cisneros-Zevallos, L. (2010). Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. *Food Chemistry*, *119*(4), 1485–1490. https://doi.org/10.1016/J.FOODCHEM.2009.09.030

Chauhan, D., Kumar, K., Thakur, P., Ahmed, N., Ul, Q., Rizvi, E. H., & Jan, S. (2022). Impact of soaking, germination, fermentation, and roasting treatments on nutritional, anti-nutritional, and bioactive composition of black soybean (Glycine max L. *Researchgate.NetD Chauhan, K Kumar, N Ahmed, P Thakur, Q Rizvi, S Jan, AN YadavJ. Appl. Biol. Biotechnol, 2022•researchgate.Net*. https://doi.org/10.7324/JABB.2022.100523

Cho, D., & Lim, S. (2016). Germinated brown rice and its bio-functional compounds. *Food Chemistry*.

Choque-Quispe, D., Ligarda-Samanez, C. A., Ramos-Pacheco, B. S., Leguía-Damiano, S., Calla-Florez, M., Zamalloa-Puma, L. M., & Colque-Condeña, L. (2021). Phenolic compounds, antioxidant capacity, and protein content of three varieties of germinated quinoa (Chenopodium quinoa Willd). *Ingeniería e Investigación*.

Donkor, O. N., Stojanovska, L., Ginn, P., Ashton, J., & Vasiljevic, T. (2012). Germinated grains – Sources of bioactive compounds. *Food Chemistry*, *135*(3), 950–959. https://doi.org/10.1016/j.foodchem.2012.05.058

Duenas, M., Hernandez, T., Estrella, I., & Fernandez, D. (2009). Germination as a process to increase the polyphenol content and antioxidant activity of lupin seeds (Lupinus angustifolius L.). *Food Chemistry*.

El-Adawy, T. A. (2002). Nutritional composition and antinutritional factors of chickpeas (Cicer arietinum L.) undergoing different cooking methods and germination. *Plant Foods for Human Nutrition*, *57*(1), 83–97. https://doi.org/10.1023/A:1013189620528

Frias, J., Diaz-Pollan, C., Hedley, C. L., & Vidal-Valverde, C. (1995). Evolution of Trypsin Inhibitor Activity during Germination of Lentils. *Journal of Agricultural and Food Chemistry*, *43*(8), 2231–2234. https://doi.org/10.1021/JF00056A049

Gharachorloo, M., Ghiassi Tarzi, B., & Baharinia, M. (2013). The Effect of Germination on Phenolic Compounds and Antioxidant Activity of Pulses. *Journal of the American Oil Chemists' Society*, *90*(3), 407–411. https://doi.org/10.1007/S11746-012-2170-3

Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R., & Panneerselvam, R. (2007). Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in Catharanthus roseus seedlings under salt stress. *South African Journal of Botany*.

Gupta, R. K., Gupta, K., Sharma, A., Das, M., Ansari, I. A., & Dwivedi, P. D. (2017). Health risks & benefits of Chickpea (Cicer arietinum) consumption. *Journal of Agricultural and Food Chemistry*, *65*(1), 6–27. https://doi.org/10.1021/ACS.JAFC.6B02629

Hung, P. Van, Maeda, T., Yamamoto, S., & Morita, N. (2012). Effects of germination on nutritional composition of waxy wheat. *Journal of the Science of Food and Agriculture*, *92*(3), 667–672. https://doi.org/10.1002/jsfa.4628

Jyothi, E., & PR, R. (1981). *The effect of germination and cooking on the in vitro digestibility of starch in some legumes*.

Khatoon, N., & Prakash, J. (2006). Nutrient retention in microwave cooked germinated legumes. *Food Chemistry*, *97*(1), 115–121. https://doi.org/10.1016/j.foodchem.2005.03.007

Koehler, P., Hartmann, G., Wieser, H., & Rychlik, M. (2007). Changes of Folates, Dietary Fiber, and Proteins in Wheat As Affected by Germination. *Journal of Agricultural and Food Chemistry*, *55*(12), 4678–4683. https://doi.org/10.1021/jf0633037

Lin, P. Y., & Lai, H. M. (2006). Bioactive compounds in legumes and their germinated products. *Journal of Agricultural and Food Chemistry*, *54*(11), 3807–3814.

López-Amorós, M., Hernández, T., & Estrella, I. (2006a). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition*.

López-Amorós, M., Hernández, T., & Estrella, I. (2006b). Effect of germination on legume phenolic compounds and their antioxidant activity. *Journal of Food Composition*.

Mamilla, R. K., & Mishra, V. K. (2017). Effect of germination on antioxidant and ACE inhibitory activities of legumes. *LWT*, *75*, 51–58. https://doi.org/10.1016/J.LWT.2016.08.036

Maphosa, Y., & Jideani, V. A. (2017). *The role of legumes in human nutrition, Functional Food-Improve Health through Adequate Food.* InTechOpen. https://doi.org/doi, 10, 66263

Masood, T., Shah, H., Plant, A. Z.-J. J. of A. &, & 2014, undefined. (2014). Effect of sprouting time on proximate composition and ascorbic acid level of mung bean (Vigna radiate L.) and chickpea (Cicer arietinum L.) seeds. *Thejaps.Org.PkT Masood, HU Shah, A ZebJAPS: Journal of Animal & Plant Sciences, 2014•thejaps.Org.Pk*.

Mierziak, J., Kostyn, K., & Kulma, A. (2014). Flavonoids as Important Molecules of Plant Interactions with the Environment. *Molecules 2014, Vol. 19, Pages 16240-16265*, *19*(10), 16240–16265. https://doi.org/10.3390/MOLECULES191016240

Milán-Noris, A., Gutiérrez-Uribe, J., & Santacruz A. (2018). Peptides and isoflavones in gastrointestinal digests contribute to the anti-inflammatory potential of cooked or germinated desi and kabuli chickpea (Cicer arietinum L.). *Food Chemistry*.

Miyahira, R. F., Lopes, J. de O., & Antunes, A. E. C. (2021). The Use of Sprouts to Improve the Nutritional Value of Food Products: A Brief Review. *Plant Foods for Human Nutrition*, *76*(2), 143–152. https://doi.org/10.1007/S11130-021-00888-6

Munteanu, I., sciences, C. A.-I. journal of molecular, & 2021, undefined. (2021). Analytical methods used in determining antioxidant activity: A review. *Mdpi.ComIG Munteanu, C ApetreiInternational Journal of Molecular Sciences, 2021•mdpi.Com*. https://doi.org/10.3390/ijms22073380

Pawar, V., Sawate, A., & Ingle, U. (1986). Changes in phytate phosphorus and minerals during germination and cooking of moth bean (Phaseolus aconitifolius Jacq) seeds. *Journal of Food Science*.

Pedranzani, H., Sierra-De-Grado, R., Vigliocco, A., Miersch, O., & Abdala, G. (2007). Cold and water stresses produce changes in endogenous jasmonates in two populations of Pinus pinaster Ait. *Plant Growth Regulation*, *52*(2), 111–116. https://doi.org/10.1007/S10725-007-9166-2

Raju, J., & Mehta, R. (2008). Cancer chemopreventive and therapeutic effects of diosgenin, a food saponin. *Nutrition and Cancer*.

Rizvi, Q., Kumar, K., Ahmed, N., & Yadav, A. (2022). Influence of soaking and germination treatments on the nutritional, anti-nutritional, and bioactive composition of pigeon pea (Cajanus cajan L.). *J. Appl. Biol. Biotechnol*.

Saleh, H., Hassan, A., Mansour, E., & Fahmy, H. (2019). Melatonin, phenolics content and antioxidant activity of germinated selected legumes and their fractions. *Journal of the Saudi Society of Agricultural Sciences*.

Sangronis, E., & Machado, C. (2007). Influence of germination on the nutritional quality of Phaseolus vulgaris and Cajanus cajan. *LWT-Food Science and Technology*.

Sierra, I., and, C. V.-J. of the S. of F., & 1999, undefined. (1999). Kinetics of free and glycosylated B6 vitamers, thiamin and riboflavin during germination of pea seeds. *Wiley Online LibraryI Sierra, C Vidal-ValverdeJournal of the Science of Food and Agriculture, 1999•Wiley Online Library*.

Sokrab, A., Ahmed, I., & Babiker, E. (2012). Effect of germination on antinutritional factors, total, and extractable minerals of high and low phytate corn (Zea mays L.) genotypes. *Journal of the Saudi Society*.

Subbulakshmi, G., Kumar, K., & Venkataraman, L. (1976). *Effect of germination on the carbohydrates, proteins, trypsin inhibitor, amylase inhibitor and hemagglutinin in horsegram and mothbean.*

Tan Khang, D., Nhan Dung, T., Abdelghany Elzaawely, A., Dang Xuan, T., Vinson, J., & Smith, C. J. (2016). Phenolic profiles and antioxidant activity of germinated legumes. *Foods*, . https://doi.org/10.3390/foods5020027

Urbano, G., López-Jurado, M., Aranda, C., Vilchez, A., Cabrera, L., Porres, J. M., & Aranda, P. (2006). Evaluation of zinc and magnesium bioavailability from pea (Pisum sativum, L.) sprouts. Effect of illumination and different germination periods. *International Journal of Food Science and Technology*, *41*(6), 618–626. https://doi.org/10.1111/J.1365-2621.2005.01107.X

Urbano, G., Lopez-Jurado, M., Hernandez, J., Fernandez, M., Moreu, M. C., Frias, J., Diaz-Pollan, C., Prodanov, M., & Vidal-Valverde, C. (1995). Nutritional Assessment of Raw, Heated, and Germinated Lentils. *Journal of Agricultural and Food Chemistry*, *43*(7), 1871–1877. https://doi.org/10.1021/JF00055A022

Vidal-Valverde, C., & Frias, J. (1992). *Changes in carbohydrates during germination of lentils*.

Vidal-Valverde, C., Frias, J., Estrella, I., Gorospe, M. J., Ruiz, R., & Bacon, J. (1994). Effect of Processing on Some Antinutritional Factors of Lentils. *Journal of Agricultural and Food Chemistry*, *42*(10), 2291–2295. https://doi.org/10.1021/JF00046A039

Vidal-Valverde, C., Frias, J., Hernández, A., Martín-Alvarez, P. J., Sierra, I., Rodríguez, C., Blazquez, I., & Vicente, G. (2003). Assessment of nutritional compounds and antinutritional factors in pea (Pisum sativum) seeds. *Journal of the Science of Food and Agriculture*, *83*(4). https://doi.org/10.1002/jsfa.1309

Vidal-Valverde, C., Frias, J., Sierra, I. (2002). New functional legume foods by germination: effect on the nutritive value of beans, lentils and peas. *SpringerC Vidal-Valverde, J Frias, I Sierra, I Blazquez, F Lambein, YH KuoEuropean Food Research and Technology*, *215*(6), 472–477. https://doi.org/10.1007/s00217-002-0602-2

SOME PHYSICOCHEMICAL PROPERTIES OF THE FRUITS AND SEEDS OF *VIBURNUM* L. SPECIES GROWING IN TURKEY

Benan DİNÇ GİRGİN[1]*, Erman DUMAN*[2*]

[1] *Kütahya Dumlupınar University, Pazarlar Vocational School, Kütahya, Turkey*

[2] *Afyon Kocatepe University, Faculty of Engineering, Department of Food Engineering, Afyonkarahisar,*

Turkey

Corresponding author e-mail: eduman@aku.edu.tr

ABSTRACT

In this study, it was aimed to determine some physicochemical properties of fruits and seeds of Viburnum L. species collected in two different years and grown naturally in different provinces of Turkey (Kayseri/Melikgazi, Ankara/Yenimahalle, Amasya/Taşova, Balıkesir/Erdek) and to determine the oil yield and mineral substance contents of the seeds and to compare between species. According to the results of the study, when the averages of the two-year data of the fruits of Viburnum species were evaluated, the averages of moisture content, ash content, dietary fiber content and protein content varied between 7,10-8,83 %, 2,44-5,64 %, 35,92- 65,03 g/100g, 2,35-3,88 %, respectively. On the other hand, when the averages of the two-year data of the seeds of Viburnum species were evaluated, seed length, seed width, thousand grain weight, fruit flesh/seed weight, moisture content, ash content, dietary fiber content, protein and fat content, respectively; 5,19- 7,52 mm, 3,89- 7,06 mm, 25,73- 35,21 g, 1,74- 6,56, 6,40- 7,54 %, 1,49- 2,88 %, 58,07- 71,62 g/100g, 7,84-10,79 %, 7,92-18,83 %, respectively. In addition, the mineral matter concentrations of the seeds of Viburnum L. species were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). It was determined that each of the species was rich in Ca, K, Mg, P minerals and also had a significant content of Al, Fe, Zn minerals.

Keywords: *Viburnum* L., Fruit, Seed, Physicochemical, Food

INTRODUCTION

The genus *Viburnum* L., belonging to the family *Adoxaceae*, comprises approximately 200 species of small trees and shrubs. These species are distributed in temperate and subtropical regions ranging from South America to Southeast Asia (Ran et al., 2020; Xiang et al., 2020). In Turkey, the genus *Viburnum* L. is represented by four species: *Viburnum opulus* L., *Viburnum lantana L.*, *Viburnum orientale Pallas*, and Viburnum tinus L. (Altun et al., 2010; Davis, 1970). The most extensively studied species include *Viburnum opulus* L., *Viburnum tinus* L., *Viburnum lantana* L., and *Viburnum orientale Pallas* (Konarska and Domaciuk, 2018; Wang et al., 2010).

The edible dark red fruits of *V. opulus* are used to produce a traditional beverage called "gilaburu" in Central Anatolia (Baytop, 1999). The fruit yield per tree is approximately 8,4 kg, and the juice yield is about 43,5% (Alizadeh et al., 2007; Davis, 1970). Gilaburu is harvested in October and November in Turkey and is used to make dried fruit, pickles, and jam (Alizadeh et al., 2007; Kalyoncu et al., 2013). Gilaburu (*Viburnum opulus* L.), primarily grown in Kayseri and its surroundings, is a fruit with high levels of vitamin C and phenolic compounds, and due to its valeric acid content, it has a strong, distinctive taste (Dinç et al., 2012). In a study conducted with genotypes of *V. opulus* in Gümüşhane province of Turkey, it was reported that the most abundant organic acids in gilaburu fruits were tartaric acid (98-114 mg/100g) and malic acid (85-130 mg/100g), while fumaric acid (7-15 mg/100g) and succinic acid (3-9 mg/100g) were found in small amounts (Ozrenk et al., 2020). Another study reported that the dry content of gilaburu fruit contained 7,81% water-soluble dry matter, 5,83% reducing sugar, 6,71% crude protein, 9,86% dietary fiber, 560 mg/kg ascorbic acid, and 402,62 mg/kg sodium (Herrera, 1987).

V. lantana is notably rich in protein, saponins, and volatile oils (Konarska and Domaciuk, 2018). In a study examining the scavenging effects of plant extracts on superoxide anion at three different concentrations (2,5, 5, and 10 mg/ml), it was found that although *V. lantana* fruit extract did not show any scavenging activity on superoxide anion at the tested concentrations, the branch extract of *V. lantana* exhibited a strong superoxide radical scavenging effect (IC50 3,1 mg/ml) (Levent Altun et al., 2008). When the essential oil compositions of *V. opulus* and *V. lantana* were examined, the main components of *V. opulus* were identified as phytol (7,8%), trans-β-damascenone (4,9%), α-cadinol (4,8%), γ-cadinene (4,7%), Δ-cadinene (4,5%), and methyl pentanoate (4,1%). In contrast, the main components of *V. lantana* were occidenol (6,3%), α-cadinol (5,6%), γ-cadinene (4,6%), 2E,4E-decadienal (4,5%), n-heptanal (3,9%), and Δ-cadinene (3,4%) (Yılmaz et al., 2008). Essential oils obtained from the dried leaves of *V. orientale* were determined to have linalool (58,50%), α-terpineol (12,63%), and geraniol (12,53%) as the main components (Yürüker et al., 1995). In a study investigating the chlorogenic acid content of *V. orientale* and *V. tinus*, it was found that the highest chlorogenic acid content (0,5069%) was in *V. orientale* fruit samples, while the lowest chlorogenic acid content (0,0141%) was in *V. tinus* branches. Chlorogenic acid has strong antioxidative and free radical scavenging activities, enhances the resistance of LDL to lipid peroxidation, and prevents DNA damage. Therefore, it has been suggested that *V. orientale* fruits can be considered a good source of chlorogenic acid (Özbilgin et al., 2015). In a study investigating the antimicrobial activities of essential oils obtained from *V. opulus*, *V. lantana*, and *V. orientale* against *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, and *B. cereus* bacteria and fungi, it was observed that the essential oils of *V. opulus* and *V. lantana* showed no antimicrobial activity against all tested microorganisms, while the essential oil of *V. orientale* exhibited weak antibacterial activity against Gram-positive bacteria *E. faecalis*, *S. aureus*, and *B. cereus* (Yılmaz et al., 2008).

Although information is available in the literature regarding the species *V. opulus*, comprehensive data on the physicochemical properties of fruits and seeds of other *Viburnum*

L. species are lacking. Accordingly, this study aimed to determine the moisture content, ash content, protein content, and dietary fiber content of fruit and seed samples of four different *Viburnum* L. species grown in Turkey, as well as some basic physical properties, crude oil content, and mineral content in seed samples. The differences between the species were identified through the analyses mentioned in the material and methods section.

MATERIAL AND METHODS

Materials

Fruit samples of four different *Viburnum* L. species used in the study were obtained over two consecutive years from four different provinces in Turkey (*V. opulus* L.-Kayseri, *V. lantana* L.-Ankara, *V. orientale Pallas*-Amasya, *V. tinus* L.-Balıkesir).

Preparation of Samples

The fruit samples of *Viburnum* L. species were weighed in the laboratory, and the seeds and fruits were separated from each other. The fruit pulp parts of the fruits from which the seeds were removed were dried. The seeds were washed with pure water, then drained and dried. All samples, once dried, were stored in cloth bags at +4 °C.

Methods

Analyses Performed on Fruits and Seeds

Ratio of Fruit Weight to Seed Weight

For each species brought to the laboratory, selected fresh fruit samples were weighed on a balance with 0,01 g accuracy, the seeds were removed, and the seed weights were determined. Then, for each sample, the ratio of fruit weight to seed weight was calculated.

Moisture Content

Dried and ground samples of 10 g of fruit and seeds were previously dried in an oven at 105 °C and weighed in desiccators until a constant weight was achieved. The moisture content of the fruit and seed samples was calculated as a percentage of weight loss. Analysis calculations were performed based on dry weight (Cemeroğlu, 2010). Other analyses were also based on dry weight according to the obtained moisture results.

$$\%\ Nem = \frac{(m_2 - m_3)}{(m_2 - m_1)} x100$$

m_1: Weight of dried empty drying container (g)

m_2: Weight of analysis sample and drying vessel (g)

m_3: Weight of the drying container with the analysis sample after drying (g)

Ash Content

Dried and ground samples of 3 g each of fruit and seeds were weighed in crucibles that had been dried and cooled, then placed in a muffle furnace at 750 °C until completely combusted. Subsequently, the ash content was calculated as a percentage (Cemeroğlu, 2007).

$$\% \, Kül = \frac{(m_2 - m_1)}{\mathrm{m}} x100$$

m_1= Weight of crucible brought to fixed weighing (g)
m_2= Crucible and ash weight after incineration (g)
m = Weight of sample taken (g)

Protein Content

Protein content in dried and ground fruit and seeds was determined using the Kjeldahl method. The amount of nitrogen (N) determined by the Kjeldahl method was multiplied by a factor of 6.25 to calculate the protein content (Kacar, 1994).

Dietary Fiber Content

Total dietary fiber content of dried and ground fruit and seeds was measured using the AOAC method (AOAC, 1985).

Analyses Performed on Seeds

Seed Size

The length and width measurements of homogeneously obtained seed samples were performed using a caliper.

Seed Weight per Thousand Seeds

Homogeneous samples of 1000 seeds were weighed on a balance with 0,01 g accuracy to determine the weight per thousand seeds (Aksoy, 1991).

Crude Fat Content

From dried and ground seed samples, 10 g were weighed into Soxhlet thimbles and placed in an extractor. 150 ml of n-hexane was added as a solvent, and the extraction was carried out for 6 hours at the solvent's boiling temperature. After the extraction process was completed, the solvent was removed, and the crude fat content was calculated based on dry weight (Ferrentino et al., 2020).

Mineral Content

From dried and ground seed samples, approximately 0,5 g samples were placed in a burning vessel, and 9 ml of pure HNO_3 and 1 ml of H_2O were added. The sample was digested in a Milestone Ethos UP microwave device at 210 °C and diluted to the desired volume with water. The concentrations were determined using ICP-AES (Agilent Technologies 7800 ICP-MS) (Skujins, 1998).

Statistical Analysis

All analyses were repeated at least twice. Comparison of the measured properties was carried out using one-way ANOVA and Tukey tests. Statistical analyses were performed using the Minitab 21 package program (Minitab, 2021).

RESEARCH FINDINGS AND DISCUSSION

In the context of this research, the physicochemical properties of the fruits of *Viburnum* L. species are given in Table 1.

Table 1. Physicochemical properties of fruits of *Viburnum* L. species

Tür	Hasat	Nem miktarı (%)	Kül miktarı (%)	Protein miktarı (%)	Diyet lif miktarı (g/100g)
Viburnum opulus L.	1. Yıl	9,47±0,39^{A}	2,80±0,24^{B}	2,22±0,08^{D}	37,05±1,63^{C}
	2. Yıl	7,22±0,11^{b}	2,07±0,12^{d}	2,71±0,00^{b}	42,79±1,82^{b}
	Ortalama	8,35±1,60$^{\mathbf{A}}$	2,44±0,52$^{\mathbf{B}}$	2,47±0,35$^{\mathbf{A}}$	39,92±4,06$^{\mathbf{B}}$
Viburnum lantana L.	1. Yıl	7,54±0,01^{B}	3,06±0,02^{B}	3,31±0,07^{B}	43,25±0,41^{B}
	2. Yıl	7,59±0,40^{b}	3,39±0,11^{b}	3,70±0,14^{a}	35,29±0,57^{c}
	Ortalama	7,57±0,04$^{\mathbf{A}}$	3,23±0,23$^{\mathbf{B}}$	3,51±0,28$^{\mathbf{A}}$	39,27±5,63$^{\mathbf{B}}$
Viburnum orientale Pallas	1. Yıl	7,08±0,15^{B}	5,73±0,50^{A}	4,39±0,08^{A}	33,74±1,22^{D}
	2. Yıl	7,12±0,26^{b}	5,54±0,02^{a}	3,37±0,28^{ab}	38,10±0,29^{c}
	Ortalama	7,10±0,03$^{\mathbf{A}}$	5,64±0,13$^{\mathbf{A}}$	3,88±0,72$^{\mathbf{A}}$	35,92±3,08$^{\mathbf{B}}$
Viburnum tinus L.	1. Yıl	8,81±0,24^{A}	2,97±0,01^{B}	2,83±0,16^{C}	65,51±1,44^{A}
	2. Yıl	8,84±0,14^{a}	2,65±0,00^{c}	1,86±0,15^{c}	64,55±2,22^{a}
	Ortalama	8,83±0,02$^{\mathbf{A}}$	2,81±0,23$^{\mathbf{B}}$	2,35±0,69$^{\mathbf{A}}$	65,03±0,68$^{\mathbf{A}}$

±: Standard deviation; A-D: Statistical comparison of species within themselves in the 1st harvest year (at the $p<0.05$ level); a-d: Statistical comparison of species within themselves in the 2nd harvest year (at the $p<0.05$ level); **A-B**: Statistical comparison of harvest year averages of species within themselves (at the $p<0.05$ level).

Table 1. presents the moisture content, ash content, protein content, and dietary fiber content of the fruits of different *Viburnum* L. species. The two-year average moisture content of the fruits of all species was found to be 8,35% for *V. opulus*, 7,57% for *V. lantana*, 7,10% for *V. orientale*, and 8,83% for *V. tinus*. The analysis results showed a significant difference in moisture levels among the *Viburnum* L. species in the first harvest year. Specifically, the moisture content of *V. opulus* and *V. tinus* was found to be higher than that of *V. lantana* and

V. orientale. Similarly, a significant difference in moisture levels was also observed among the *Viburnum* species in the second harvest year. It was determined that the moisture content of *V. tinus* was higher than that of *V. opulus, V. lantana*, and *V. orientale* species. Previous studies in the literature have reported the moisture content of fresh *V. opulus* fruits. The moisture values presented in Table 1. reflect the results of moisture analysis conducted on dried fruits. According to the literature, Akbulut et al. (2008) reported the moisture content of fresh *V. opulus* fruits as 88,2%, while Cam et al. (2007) reported it as 89,16%. Therefore, the results obtained in our study cannot be directly compared with those in the literature because our analysis was conducted on dried fruits of *Viburnum* L. species.

The two-year average ash content of the fruits of all species was determined to be 2,44% for *V. opulus*, 3,23% for *V. lantana*, 5,64% for *V. orientale*, and 2,81% for *V. tinus*. In the first harvest year, a significant difference was observed in the ash content among the *Viburnum* L. species. Specifically, *V. orientale* had a higher ash content compared to the other species. Similarly, in the second harvest year, a significant difference in ash content was also observed among the *Viburnum* L. species. The highest ash content was found in *V. orientale*, while the lowest was in *V. opulus*. Previous studies in the literature have reported the ash content of the fruits of *V. opulus* species. According to these studies, the ash content of *V. opulus* fruits was reported as 0,38% by Cam et al. (2007), 0,11% by Kalyoncu et al. (2013), and 1,28% by Akbulut et al. (2008). Compared to these reported results, the ash content of *V. opulus* fruits was found to be higher in our study.

The two-year average protein content of the fruits of all species was determined to be 2,47% for *V. opulus*, 3,51% for *V. lantana*, 3,88% for *V. orientale*, and 2,35% for *V. tinus*. In the first harvest year, a significant difference was observed in the protein content among the *Viburnum* L. species. Specifically, the highest protein content was found in *V. orientale*, while the lowest was in *V. opulus*. Similarly, in the second harvest year, a significant difference in protein content was also observed among the *Viburnum* L. species. It was determined that *V. tinus* had a lower protein content compared to the other species. Previous studies in the literature have only reported the protein content of *V. opulus* fruits. Ozrenk et al. (2020) reported that the protein content of the *V. opulus* genotypes they studied ranged between 5,67% and 6,71%. Akbulut et al. (2008) reported the protein content of *V. opulus* fruits as 6,48%. In our study, a lower protein content was found compared to these two studies. However, compared to another study conducted by Kalyoncu et al. (2013), which reported the protein content of *V. opulus* fruits in Turkey as 0,2%, a higher protein content was found in our study.

The two-year average dietary fiber content of the fruits of all species was determined to be 39,92 g/100g for *V. opulus*, 39,27 g/100g for *V. lantana*, 35,92 g/100g for *V. orientale*, and 65,03 g/100g for *V. tinus*. In the first harvest year, a significant difference was observed in the dietary fiber content among the *Viburnum* L. species. Specifically, the highest dietary fiber content was found in *V. tinus*, while the lowest was in *V. orientale*. Similarly, in the second harvest year, a significant difference in dietary fiber content was also observed among the *Viburnum* L. species. The highest dietary fiber content was found in *V. tinus*, while the lowest dietary fiber contents were found in *V. lantana* and *V. orientale*. Akbulut et al. (2008) reported the crude cellulose content of *V. opulus* fruits as 18,07%, and Kalyoncu et al. (2013) reported the crude fiber content as 6,56%. In a comprehensive morphological and biochemical characterization study of 14 *V. opulus* L. genotypes collected from Gümüşhane province in Turkey, the crude cellulose content of all *V. opulus* genotypes was reported to range between 16,6% and 18,7% (Ozrenk et al., 2020). According to the literature, the dietary fiber content of the *V. opulus* species was found to be higher.

Table 2.1. and Table 2.2. show the physicochemical properties of the seeds of *Viburnum* L. species.

Table 2.1. Physicochemical properties of seeds of *Viburnum* L. species

Tür	Hasat	Çekirdek boyu (mm)	Çekirdek eni (mm)	Bindane ağırlığı (g)	Meyve ağırlığı/ Çekirdek ağırlığı	Nem miktarı (%)
Viburnum opulus L.	1.Yıl	7,65±0,63^{A}	7,14±0,35^{A}	37,00±2,37^{AB}	6,26±1,06^{A}	6,25±0,09^{C}
	2.Yıl	7,38±0,66^{ab}	6,98±0,40^{a}	33,41±0,54^{a}	6,89±2,1^{a}	6,55±0,09^{a}
	Ortalama	7,52±0,19$^{\mathbf{A}}$	7,06±0,11$^{\mathbf{A}}$	35,21±2,54$^{\mathbf{A}}$	6,56±0,45$^{\mathbf{A}}$	6,40±0,21$^{\mathbf{A}}$
Viburnum lantana L.	1.Yıl	6,40±0,58^{B}	4,72±0,27^{C}	24,22±2,93^{C}	2,69±0,47^{B}	7,44±0,16^{AB}
	2.Yıl	6,83±0,73^{b}	5,03±0,5^{b}	27,24±0,90^{d}	4,19±0,49^{b}	7,50±0,31^{a}
	Ortalama	6,62±0,30$^{\mathbf{AB}}$	4,88±0,22$^{\mathbf{C}}$	25,73±2,14$^{\mathbf{A}}$	3,44±1,06$^{\mathbf{B}}$	7,47±0,04$^{\mathbf{A}}$
Viburnum orientale Pallas	1.Yıl	7,21±0,40^{A}	5,94±0,34^{B}	38,74±3,34^{A}	2,91±0,34^{B}	7,17±0,16^{B}
	2.Yıl	7,67±0,43^{a}	5,60±0,62^{b}	31,63±0,54^{b}	3,24±0,18^{b}	6,47±0,77^{a}
	Ortalama	7,44±0,33$^{\mathbf{A}}$	5,77±0,24$^{\mathbf{B}}$	35,19±5,03$^{\mathbf{A}}$	3,08±0,23$^{\mathbf{B}}$	6,82±0,50$^{\mathbf{A}}$
Viburnum tinus L.	1.Yıl	5,74±0,79^{B}	4,06±0,40^{D}	34,27±3,3^{B}	1,60±0,79^{C}	7,71±0,14^{A}
	2.Yıl	4,64±0,62^{c}	3,71±0,40^{c}	29,91±0,88^{c}	1,88±0,27^{c}	7,36±0,15^{a}
	Ortalama	5,19±0,78$^{\mathbf{B}}$	3,89±0,25$^{\mathbf{D}}$	32,09±3,08$^{\mathbf{A}}$	1,74±0,20$^{\mathbf{B}}$	7,54±0,25$^{\mathbf{A}}$

±: Standard deviation; A-D: Statistical comparison of species within themselves in the 1st harvest year (at the $p<0.05$ level); a-d: Statistical comparison of species within themselves in the 2nd harvest year (at the $p<0.05$ level); **A-D**: Statistical comparison of harvest year averages of species within themselves (at the $p<0.05$ level).

Table 2.1. presents the seed length, seed width, thousand seed weight, fruit weight/seed weight ratio, and moisture content of *Viburnum* L. species. The two-year averages of seed lengths for all species were determined as follows: *V. opulus* 7,52 mm, *V. lantana* 6,62 mm, *V. orientale* 7,44 mm, and *V. tinus* 5,19 mm. A significant difference was observed among *Viburnum* L. species in terms of seed length during the first harvest year. When examining this significant difference, it was found that the seed lengths of *V. opulus* and *V. orientale* were larger than those of *V. lantana* and *V. tinus*. A significant difference was also observed in the second harvest year in terms of seed length. In this significant difference, the species with the highest seed length was *V. orientale*, and the species with the lowest seed length was *V. tinus*. The two-year averages of seed widths for all species were determined as follows: *V. opulus* 7,06 mm, *V. lantana* 4,88 mm, *V. orientale* 5,77 mm, and *V. tinus* 3,89 mm. A significant difference was observed among *Viburnum* L. species in terms of seed width during both the

first and second harvest years. When examining this significant difference, it was found that the species with the highest seed width in both harvest years was *V. opulus*, and the species with the lowest seed width was *V. tinus*. In terms of shape, *V. tinus* has a spherical structure, while the other species have a flattened and oval structure. In the literature, Kalyoncu et al. (2013) reported the average seed length and width of *V. opulus* as 7,76 mm and 7,67 mm, respectively. These values are consistent with our study. Kaliniewicz and Choszcz (2021), in their study on different *Viburnum* L. species, reported the seed length and width of *V. lantana* as 9,89 mm and 6,90 mm, respectively. According to these reported values, the seed length and width in our study were found to be lower.

The two-year averages of the thousand-seed weights for all species were determined as follows: *V. opul*us 35,21 g, *V. lantana* 25,73 g, *V. orientale* 35,19 g, and *V. tinus* 32,09 g. A significant difference was observed among *Viburnum* L. species in terms of thousand-seed weight during the first harvest year. When examining this significant difference, it was found that the species with the highest thousand-seed weight was *V. orientale*, and the species with the lowest thousand-seed weight was *V. lantana*. A significant difference was also observed in the second harvest year in terms of thousand-seed weight. In this significant difference, the species with the highest thousand-seed weight was *V. opulus*, and the species with the lowest thousand-seed weight was *V. lantana*. In the literature, Alizadeh et al. (2007) reported the thousand-seed weight of *V. opulus* as 76,57 g in terms of wet weight. Keskin and Kaya (2011) stated that the weight of a single seed of *V. opulus* ranges between 0,104-0,112 g in terms of wet weight. Kaliniewicz and Choszcz (2021) reported that the weight of a single seed of *V. lantana* ranges between 32-37 mg in terms of dry weight, while the weight of a single seed of *V. opulus* ranges between 27-32 mg. These values are consistent with the values determined in our study.

The two-year averages of the ratios of fruit weights to seed weights for all species were determined as follows: *V. opulus* 6,56, *V. lantana* 3,44, *V. orientale* 3,08, and *V. tinus* 1,74. A significant difference was observed among *Viburnum* L. species in terms of the ratio of fruit weight to seed weight during both the first and second harvest years. When examining this significant difference, it was found that the species with the highest ratio of fruit weight to seed weight in both harvest years was *V. opulus*, and the species with the lowest ratio was *V. tinus*. Upon reviewing the literature, no studies were found that reported the ratio of fruit weight to seed weight for *Viburnum* L. species, but values for the wet weight of a single seed and a single fruit of *V. opulus* were encountered. Apart from the ratio of fruit weight to seed weight mentioned in Table 2.1., the fresh fruit and seed weights in terms of wet weight for all *Viburnum* L. species were also determined. In this context, the two-year averages of the fresh single fruit weights of *Viburnum* L. species were determined as follows: *V. opulus* 0,60 g, *V. lantana* 0,19 g, *V. orientale* 0.,32 g, and *V. tinus* 0,15 g The two-year averages of the fresh single seed weights of *Viburnum* L. species were determined as follows: *V. opulus* 0,09 g, *V. lantana* 0,05 g, *V. orientale* 0,11 g, and *V. tinus* 0,08 g. Ozrenk et al. (2020) reported that the fresh fruit weights of *V. opulus* range between 0,53-0,86 g, while Keskin and Kaya (2011) indicated that they range between 0,765-0,768 g Kalyoncu et al. (2013) reported the fresh single fruit weight of *V. opulus* as 0,87 g, and Akbulut et al. (2008) reported it as 0,65 g Kalyoncu et al. (2013) also reported the single seed weight of *V. opulus* as 0,08 g in terms of wet weight. These reported values are consistent with the values determined in our study.

The two-year averages of the moisture content of seeds for all species were determined as follows: *V. opulus* 6,40%, *V. lantana* 7,47%, *V. orientale* 6,82%, and *V. tinus* 7,54%. A significant difference was found among *Viburnum* L. species in terms of seed moisture content during the first harvest year. When examining this significant difference, it was found that the moisture content of *V. tinus* was higher than that of the other species. In the second harvest year, no significant difference was found in terms of seed moisture content. In the literature, Cam et al. (2007) reported the moisture content of seeds in fresh *V. opulus* fruits as 9,31%. Yunusova et al. (2004) examined some changes in *V. opulus* seeds during storage under natural conditions and reported that the seed moisture content ranged between 5,3% and 9,3%. These reported values are consistent with the values determined in our study.

Table 2.2. Physicochemical properties of seeds of *Viburnum* L. species (continuation of Table 2.1.)

Tür	Hasat	Kül miktarı (%)	Protein miktarı (%)	Ham yağ miktarı (%)	Diyet lif miktarı (g/100g)
Viburnum opulus L.	1.Yıl	1,95±0,01^{C}	10,72±0,49^{AB}	12,96±0,10^{C}	60,11±0,41^{B}
	2.Yıl	1,67±0,03^{c}	10,81±0,71^{a}	13,28±0,28^{b}	67,11±0,48^{a}
	Ortalama	1,81±0,20$^{\mathbf{BC}}$	10,76±0,06$^{\mathbf{A}}$	13,12±0,23$^{\mathbf{B}}$	63,61±4,95$^{\mathbf{A}}$
Viburnum lantana L.	1.Yıl	1,50±0,02^{D}	7,59±0,04^{B}	14,22±0,14^{B}	78,02±2,37^{A}
	2.Yıl	1,47±0,03^{d}	8,09±0,04^{c}	13,56±2,06^{b}	65,21±0,91^{a}
	Ortalama	1,49±0,02$^{\mathbf{C}}$	7,84±0,35$^{\mathbf{B}}$	13,89±0,47$^{\mathbf{B}}$	71,62±9,06$^{\mathbf{A}}$
Viburnum orientale Pallas	1.Yıl	2,24±0,03^{B}	10,38±0,09^{AB}	18,82±0,33^{A}	56,44±0,69^{B}
	2.Yıl	2,25±0,05^{b}	8,98±0,06^{bc}	18,83±0,30^{a}	61,64±1,10^{b}
	Ortalama	2,25±0,01$^{\mathbf{B}}$	9,68±1,00$^{\mathbf{AB}}$	18,83±0,01$^{\mathbf{A}}$	59,04±3,68$^{\mathbf{A}}$
Viburnum tinus L.	1.Yıl	3,03±0,07^{A}	11,21±1,55^{A}	7,76±0,12^{D}	55,95±2,60^{B}
	2.Yıl	2,72±0,03^{a}	10,37±0,06^{ab}	8,08±0,21^{c}	60,18±1,28^{b}
	Ortalama	2,88±0,22$^{\mathbf{A}}$	10,79±0,59$^{\mathbf{A}}$	7,92±0,23$^{\mathbf{C}}$	58,07±2,99$^{\mathbf{A}}$

±: Standard deviation; A-D: Statistical comparison of species within themselves in the 1st harvest year (at the $p<0.05$ level); a-d: Statistical comparison of species within themselves in the 2nd harvest year (at the $p<0.05$ level); **A-C**: Statistical comparison of harvest year averages of species within themselves (at the $p<0.05$ level).

Table 2.2. presents the ash content, protein content, crude fat content, and dietary fiber content of the seeds of *Viburnum* L. species. The two-year averages of the ash content of seeds for all species were determined as follows: *V. opulus* 1,81%, *V. lantana* 1,49%, *V. orientale* 2,25%, and *V. tinus* 2,88%. A significant difference was found among *Viburnum* L. species in terms of ash content during both the first and second harvest years. When examining this difference, it was found that the species with the highest ash content in both years was *V. tinus*, while the species with the lowest ash content was *V. lantana*. In the literature, Elagamey et al. (2013) reported that the ash content of grape seeds in certain varieties ranges between 2,52-2,68%. Polka et al. (2019) studied *V. opulus* in terms of its fruit, flower, and bark, and found

the ash content to be 2,96 g/100g, 4,07 g/100g, and 9,32 g/100g in dry weight, respectively. In the literature, the ash content of *V. opulus* seeds was reported only by Cam et al. (2007), who stated that the ash content of *V. opulus* seeds was 1,68%. The values obtained in our study are consistent with those reported in the literature.

The two-year averages of the protein content of the seeds for all species were determined as follows: *V. opulus* 10,76%, *V. lantana* 7,84%, *V. orientale* 9,68%, and *V. tinus* 10,79%. A significant difference was found among *Viburnum* L. species in terms of protein content during the first harvest year. When examining this difference, it was found that the protein content of *V. lantana* was lower compared to the other species. A significant difference was also found among *Viburnum* L. species in terms of protein content during the second harvest year. Again, the protein content of *V. lantana* was found to be lower compared to the other species, while the highest protein content was observed in *V. opulus*. In the literature, Yunusova et al. (2004) examined some changes in the seeds of *V. opulus* during storage under natural conditions and reported that the protein content in the seeds ranged between 4,9% and 6,0%. Elagamey et al. (2013) reported that the protein content of grape seeds in certain varieties ranged between 6,51% and 7,71%. Yan et al. (2020) reported that the seeds of *Cinnamomum camphora* contained 19,34% protein. In the literature, only the protein content of *V. opulus* among the species studied in our research was reported, and when compared with our findings, the values obtained in our study were found to be slightly higher.

The two-year averages of the crude fat content of the seeds for all species were determined as follows: *V. opulus* 13,12%, *V. lantana* 13,89%, *V. orientale* 18,83%, and *V. tinus* 7,92%. Significant differences were found among *Viburnum* L. species in terms of crude fat content during the first harvest year. When examining these differences, the species with the highest crude fat content was *V. orientale*, and the species with the lowest fat content was *V. tinus*. Significant differences were also found among *Viburnum* L. species in terms of crude fat content during the second harvest year. When examining these differences, it was found that the crude fat content of *V. opulus* and *V. lantana* was lower than that of *V. orientale* but higher than that of *V. tinus*. In the literature, Yunusova et al. (2004) examined the fat content in the dry weight of *V. opulus* seeds during eleven months of storage under natural conditions and reported fat content ranging from 12,1% to 15,1%. Cam et al. (2007) reported the fat content of *V. opulus* seeds as 13,88%. In a study on the seed oil of a different fruit, the Zivzik pomegranate, an average fat content of 19,48% was obtained through Soxhlet extraction (Yeniçeri et al., 2020). The crude fat content obtained in our study for the *V. opulus* species is consistent with the results of studies reported in the literature.

The two-year averages of the dietary fiber content of the seeds for all species were determined as follows: *V. opulus* 63,61 g/100g, *V. lantana* 71,62 g/100g, *V. orientale* 59,04 g/100g, and *V. tinus* 58,07 g/100g. Significant differences were found among *Viburnum* L. species in terms of dietary fiber content during the first harvest year. When examining these differences, it was found that the dietary fiber content of *V. lantana* was higher compared to all other species. Significant differences were also found among *Viburnum* L. species in terms of dietary fiber content during the second harvest year. When examining these differences, it was found that the dietary fiber content of *V. opulus* and *V. lantana* was higher compared to the dietary fiber content of *V. orientale* and *V. tinus*. In the literature, Kalyoncu et al. (2013) reported 6,56% crude fiber in the seeds of *V. opulus*. Kamel et al. (1985) reported the dietary fiber content of watermelon (*Citrullus vulgaris*) and grape (*Vitis vinifera*) seeds as 47,7% and 38,6%, respectively. The results obtained in our study were found to be higher than those reported in the literature.

Tables 3.1. and 3.2. show the mineral content of *Viburnum* L. species seeds and the average harvest years of mineral content of *Viburnum* L. species seeds.

Table 3.1. Mineral content of *Viburnum* L. species seeds and the average harvest years of mineral content

Mineral /Tür	**Viburnum opulus L**		**Viburnum lantana L**		**Viburnum orientale Pallas**		**Viburnum tinus L**	
	1.Yıl	**2.Yıl**	**1.Yıl**	**2.Yıl**	**1.Yıl**	**2.Yıl**	**1.Yıl**	**2.Yıl**
Li	26,32±3,28^{B}	11,00±3,86^{bc}	70,95±9,37^{A}	192,46±8,97^{a}	-	-	6,81±2,37	27,03±7,69^{b}
	Ort: 18.66±10,83$^{\mathbf{A}}$		**Ort:** 131,71±85,90$^{\mathbf{A}}$		**Ort: -**		**Ort:** 16,92±14,30$^{\mathbf{A}}$	
Be	-	0,53±0,44^{c}	2,64±2,16^{A}	8,81±5,69^{a}	2,54±1,42^{A}	2,82±1,01^{bc}	4,89±4,82^{A}	6,44±3,24^{ab}
	Ort: 0,27±0,38$^{\mathbf{A}}$		**Ort:** 5,73±4,36$^{\mathbf{A}}$		**Ort:** 2,68±0,20$^{\mathbf{A}}$		**Ort:** 5,67±1,10$^{\mathbf{A}}$	
B	14428,3±7,81^{B}	13793,51±5,86^{c}	9007,44±2,53^{C}	10384,38±4,20^{d}	19440,16±7,14^{A}	19353,12±8,29^{b}	17045,12±4,57^{AB}	21382,16±3,12^{a}
	Ort: 14110,91±449,00$^{\mathbf{AB}}$		**Ort:** 9695,91±974,00$^{\mathbf{B}}$		**Ort:** 19396,64±61,50$^{\mathbf{A}}$		**Ort:**19213,64±3067,00$^{\mathbf{A}}$	
Na	291217,69±4,33^{B}	298153,76±5,79^{b}	204868,85±1,32^{C}	323300,46±1,30^{b}	456271,69±9,85^{A}	443393,33±8,48^{a}	259673,05±3,89^{B}	403050,04±5,42^{a}
	Ort: 294685,73±4905,00$^{\mathbf{A}}$		**Ort:** 264084,66±83744,00$^{\mathbf{A}}$		**Ort:** 449832,51±9106,00$^{\mathbf{A}}$		**Ort:** 331361,55±101383$^{\mathbf{A}}$	
Mg	2878570,83±1,87^{A}	2873581,36±1,72^{ab}	1943397,56±1,54^{B}	1890828,56±5,95^{c}	2778682,44±1,23^{A}	2513033,84±4,29^{b}	2616697,97±0,95^{A}	3213161,18±1,85^{a}
	Ort: 2876076,10±3528,00$^{\mathbf{A}}$		**Ort:** 1917113,06±37172,00$^{\mathbf{B}}$		**Ort:** 2645858,14±187842,00$^{\mathbf{AB}}$		**Ort:** 2914929,58±412763,00$^{\mathbf{A}}$	
Al	19954,75±3,42^{C}	8932,02±5,62^{d}	40502,76±1,98^{B}	403270,03±3,37^{a}	80771,44±3,52^{A}	72323,48±0,86^{b}	10676,34±3,11^{D}	53428,06±0,16^{c}
	Ort: 14443,39±7794,00$^{\mathbf{A}}$		**Ort:** 221886,40±256515,00$^{\mathbf{A}}$		**Ort:** 76547,46±5974,00$^{\mathbf{A}}$		**Ort:** 32052,20±30230,00$^{\mathbf{A}}$	
P	3142255,88±6,44^{B}	3023787,93±5,50^{a}	2157525,91±5,24^{C}	1981048,79±2,16^{b}	3781977,82±2,28^{A}	3542717,26±3,72^{a}	2254034,39±3,22^{C}	3029883,21±3,11^{a}
	Ort: 3083021,91		**Ort:** 2069287,35		**Ort:** 3662347,54		**Ort:** 2641958,80	

K	8945292,33±1,44^{C}	6068977,42±2,66^{c}	5381062,89±0,89^{D}	6025798,64±1,57^{c}	11128949,29±2,50^{B}	10829049,09±1,29^{b}	12623684,79±1,61^{A}	15997284,87±1,33^{a}
	Ort: 7507134,88±2033862,00$^{\mathbf{B}}$		**Ort:** 5703430,77±455897,00$^{\mathbf{B}}$		**Ort:** 10978999,20±212061,00$^{\mathbf{AB}}$		**Ort:** 14310484,83±2385495,00$^{\mathbf{A}}$	
Ca	1067891,54±1,55^{B}	1127179,32±1,57^{b}	884616,33±1,36^{B}	886650,08±1,33^{b}	1151845,93±1,05^{B}	996040,30±0,61^{b}	1615509,08±0,24^{A}	1524445,90±1,13^{a}
	Ort: 1097535,43±41923,00$^{\mathbf{B}}$		**Ort:** 885633,21±1438,00$^{\mathbf{B}}$		**Ort:** 1073943,12±110171,00$^{\mathbf{B}}$		**Ort:** 1569977,49±64391,00$^{\mathbf{A}}$	
V	6,56±0,85^{C}	134,49±3,17^{b}	75,67±2,06^{A}	678,41±1,76^{a}	57,34±2,64^{B}	55,43±4,59^{c}	5,27±0,60^{C}	22,64±1,66^{d}
	Ort: 70,53±90,50$^{\mathbf{A}}$		**Ort:** 377,04±426,00$^{\mathbf{A}}$		**Ort:** 56,39±1,35$^{\mathbf{A}}$		**Ort:** 13,96±12,28$^{\mathbf{A}}$	
Cr	196,51±1,51^{C}	830,78±1,96^{b}	757,15±2,17^{A}	2540,59±1,02^{a}	586,50±0,41^{B}	798,54±0,55^{c}	105,10±4,26^{D}	261,45±1,66^{d}
	Ort: 513,65±448,00$^{\mathbf{A}}$		**Ort:** 1648,87±1261,00 $^{\mathbf{A}}$		**Ort:** 692,52±150,00 $^{\mathbf{A}}$		**Ort:** 183,28±110,60 $^{\mathbf{A}}$	
Mn	11388,03±0,28^{C}	11966,06±1,00^{d}	13735,82±1,23^{C}	20791,93±0,45^{c}	55986,30±3,20^{B}	52090,16±5,65^{b}	130862,46±2,82^{A}	103010,70±2,28^{a}
	Ort: 11677,05±409,00$^{\mathbf{C}}$		**Ort:** 17263,88±4989,00$^{\mathbf{BC}}$		**Ort:** 54038,23±2755,00$^{\mathbf{B}}$		**Ort:** 116936,58±19694,00$^{\mathbf{A}}$	
Fe	62269,00±0,74^{A}	82185,00±1,03^{b}	51217,32±1,59^{B}	303678,63±1,01^{a}	63927,40±1,97^{A}	31759,55±1,50^{c}	28810,87±3,73^{C}	79723,70±2,59^{b}
	Ort: 72227,00±14083,00$^{\mathbf{A}}$		**Ort:** 177447,98±178517,00$^{\mathbf{A}}$		**Ort:** 47843,48±22746,00$^{\mathbf{A}}$		**Ort:** 54267,29±36001$^{\mathbf{A}}$	
	Ort: 33,55±25,90$^{\mathbf{A}}$		**Ort:** 98,90±15,30$^{\mathbf{A}}$		**Ort:** 50,36±28,10$^{\mathbf{A}}$		**Ort:** 155,80±138,60$^{\mathbf{A}}$	
Co	36,10±5,54^{B}	27,66±3,81^{d}	39,21±4,23^{B}	211,40±1,32^{a}	30,48±2,93^{B}	67,54±3,86^{c}	186,35±1,74^{A}	126,42±3,80^{b}
	Ort: 31,88±5,97$^{\mathbf{A}}$		**Ort:** 125,31±121,80$^{\mathbf{A}}$		**Ort:** 49,01±26,20$^{\mathbf{A}}$		**Ort:** 156,39±42,40$^{\mathbf{A}}$	
Ni	6903,60±0,08^{B}	2486,72±2,13^{c}	1280,53±2,69^{D}	2623,58±1,57^{b}	8037,04±0,04^{A}	8152,47±1,26^{a}	1542,36±2,94^{C}	1652,58±2,95^{d}
	Ort: 4695,16±3123,00$^{\mathbf{A}}$		**Ort:** 1952,06±950,00$^{\mathbf{A}}$		**Ort:** 8094,76±81,60$^{\mathbf{A}}$		**Ort:** 1597,47±77,90$^{\mathbf{A}}$	

Table 3.1. (Continued)

Mineral /Tür	**Viburnum opulus L**		**Viburnum lantana L**		**Viburnum orientale Pallas**		**Viburnum tinus L**	
	1.Yıl	**2.Yıl**	**1.Yıl**	**2.Yıl**	**1.Yıl**	**2.Yıl**	**1.Yıl**	**2.Yıl**
Cu	11620,48 ±0,48^{A}	10963,41 ±1,08^{b}	8629,65± 1,87^{A}	9280,02 ± 0,12^{c}	9704,31± 1,27^{A}	10322,10 ±0,33^{bc}	10958,83± 1,27^{A}	14015,72 ±0,46^{a}
	Ort: 11291,95±465,00$^{\mathbf{A}}$		**Ort:** 8954,84±460,00$^{\mathbf{A}}$		**Ort:** 10013,21±437,00$^{\mathbf{A}}$		**Ort:** 12487,28±2162,00$^{\mathbf{A}}$	
Zn	28790,50 ±1,80^{A}	18682,60 ±1,92^{c}	16391,95 ±0,06^{C}	14477,28±0,17^{d}	23559,69± 1,73^{B}	20736,08 ±0,77^{b}	21162,77± 0,34^{B}	25538,83 ±0,93^{a}
	Ort: 23736,55±7147,00$^{\mathbf{A}}$		**Ort:** 15434,62±1354,00$^{\mathbf{A}}$		**Ort:** 22147,89±1997,00$^{\mathbf{A}}$		**Ort:** 23350,80±3094,00$^{\mathbf{A}}$	
Ga	7,39±0,77^{B}	3,70±0,08^{c}	10,87±0,33^{A}	90,78±5,53^{a}	7,03±0,21^{B}	6,78±3,52^{bc}	3,76±0,41^{C}	12,32±0,89^{b}
	Ort: 5,55 ±2,61$^{\mathbf{A}}$		**Ort:** 50,83±56,50$^{\mathbf{A}}$		**Ort:** 6,91±0,18$^{\mathbf{A}}$		**Ort:** 8,04±6,05$^{\mathbf{A}}$	
As	57,54±5,00^{A}	48,38±6,74^{b}	18,83±2,63^{B}	79,79±6,22^{a}	20,62±4,48^{B}	15,06±3,41^{d}	21,03±3,37^{B}	33,59±1,62^{c}
	Ort: 52,96±6,48$^{\mathbf{A}}$		**Ort:** 49,31±43,10$^{\mathbf{A}}$		**Ort:** 17,84±3,93$^{\mathbf{A}}$		**Ort:** 27,31±8,88$^{\mathbf{A}}$	
Se	48,10±9,69^{B}	-	8,30±4,38^{C}	56,50±6,16^{a}	91,10±10,69^{A}	65,80±3,16^{a}	7,98±1,88^{C}	-
	Ort: 24,05±34,00$^{\mathbf{A}}$		**Ort:** 32,40±34,10$^{\mathbf{A}}$		**Ort:** 78,45±17,90$^{\mathbf{A}}$		**Ort:** 3,99±5,64$^{\mathbf{A}}$	
Rb	14583,61 ±0,22^{A}	7509,01± 4,08^{b}	1280,64± 4,80^{D}	1517,06 ± 1,16^{c}	3157,87± 5,27^{C}	1410,79± 2,21^{c}	12930,79± 4,58^{B}	19466,17 ±2,25^{a}
	Ort: 11046,31±5002,00$^{\mathbf{AB}}$		**Ort:** 1398,85±167,00$^{\mathbf{B}}$		**Ort:** 2284,33±1235,00$^{\mathbf{AB}}$		**Ort:** 16198,48±4621,00$^{\mathbf{A}}$	
Sr	19327,98 ±5,32^{B}	15959,45 ±2,51^{c}	18545,83 ±2,92^{B}	24463,54±1,93^{b}	20170,70± 3,20^{B}	14338,19 ±2,43^{c}	34992,51± 1,85^{A}	34662,64 ±2,53^{a}
	Ort: 17643,72±2382,00$^{\mathbf{B}}$		**Ort:** 21504,69±4184,00$^{\mathbf{B}}$		**Ort:** 17254,45±4124,00$^{\mathbf{B}}$		**Ort:** 34827,58±233,00$^{\mathbf{A}}$	
Pd	13,19±3,55^{B}	6,94±1,38^{c}	25,38±2,60^{A}	23,94±2,02^{b}	11,55±4,19^{B}	5,92±1,78^{c}	35,97±3,25^{A}	41,62±3,81^{a}
	Ort: 10,07±4,42$^{\mathbf{BC}}$		**Ort:** 24,66±1,02$^{\mathbf{AB}}$		**Ort:** 8,74±3,98$^{\mathbf{C}}$		**Ort:** 38,80±4,00$^{\mathbf{A}}$	
Ag	207,98± 1,23^{B}	82,09±2,29^{c}	-	-	45,83±3,13^{C}	2325,61± 5,63^{b}	3238,64± 2,13^{A}	3388,03± 3,85^{a}
	Ort: 145,04±89,00$^{\mathbf{AB}}$		**Ort:** 0		**Ort:** 1185,72±1612,00$^{\mathbf{AB}}$		**Ort:** 3313,34±73,80$^{\mathbf{A}}$	
Cd	313,76± 3,29^{C}	590,66± 4,59^{bc}	-	295,16± 2,81^{c}	1373,58± 3,73^{A}	892,56±3,15^{b}	924,19±0,92^{B}	1342,07± 2,64^{a}
	Ort: 452,21±		**Ort:** 147,58		**Ort:** 1133,07		**Ort:**1133,13	
Sn	24330,35 ±3,99^{B}	21165,31 ±2,91^{b}	21439,69 ±3,02^{B}	-	55311,13± 4,90^{A}	34368,91 ±1,84^{a}	-	24314,43 ±4,37^{b}
	Ort: 22747,83±2238,00$^{\mathbf{A}}$		**Ort:** 10719,85±15160,00$^{\mathbf{A}}$		**Ort:** 44840,02±14808,00$^{\mathbf{A}}$		**Ort:** 12157,22±17193,00$^{\mathbf{A}}$	
Sb	99,54±5,51^{A}	8,84±1,88^{a}	13,87±3,48^{C}	5,66±1,52^{a}	67,85±2,34^{B}	5,37±1,47^{a}	8,57±1,39^{C}	6,77±1,83^{a}
	Ort: 54,19±64,10$^{\mathbf{A}}$		**Ort:** 9,77±5,81$^{\mathbf{A}}$		**Ort:** 36,61±44,20$^{\mathbf{A}}$		**Ort:** 7,67±1,27$^{\mathbf{A}}$	

Cs	86,91±5,04^{A}	46,21±2,02^{a}	3,64±1,95^{C}	30,27±1,04^{b}	6,99±1,76^{BC}	1,98±1,20^{c}	11,46±1,72^{B}	28,44±2,07^{b}
	Ort: 66,56±28,80$^{\mathbf{A}}$		**Ort:** 16,96±18,80$^{\mathbf{A}}$		**Ort:** 4,49±3,54$^{\mathbf{A}}$		**Ort:** 19,95±12,01$^{\mathbf{A}}$	
Ba	6168,74±2,01^{C}	8955,08±2,22^{b}	17616,04±4,53^{B}	25178,12±2,77^{a}	26706,91±2,25^{A}	27834,84±5,59^{a}	23130,01±5,75^{A}	22648,88±4,80^{a}
	Ort: 7561,91±1970,00$^{\mathbf{B}}$		**Ort:** 21397,08±5347,00$^{\mathbf{A}}$		**Ort:** 27270,88±798,00$^{\mathbf{A}}$		**Ort:** 22889,45±340,00$^{\mathbf{A}}$	
Hg	-	0,91±0,40^{b}	0,52±0,54^{B}	0,51±0,34^{b}	-	-	2,01±0,21^{A}	4,90±1,07^{a}
	Ort: 0,46±0,64$^{\mathbf{A}}$		**Ort:** 0,52±0,01$^{\mathbf{A}}$		**Ort:** 0		**Ort:** 3,46±2,04$^{\mathbf{A}}$	
Pb	15,21±2,72^{D}	51,88±5,04^{b}	88,08±3,38^{B}	109,72±4,53^{a}	70,24±4,61^{C}	30,47±4,28^{c}	253,78±2,84^{A}	57,81±2,09^{b}
	Ort: 33,55±25,90$^{\mathbf{A}}$		**Ort:** 98,90±15,30$^{\mathbf{A}}$		**Ort:** 50,36±28,10$^{\mathbf{A}}$		**Ort:** 155,80±138,60$^{\mathbf{A}}$	

±: Standard deviation; A-D: Statistical comparison of species within themselves in the 1st harvest year (at the $p<0.05$ level); a-d: Statistical comparison of species within themselves in the 2nd harvest year (at the $p<0.05$ level); A-C: Statistical comparison of harvest year averages of species within themselves (at the $p<0.05$ level); -: Could not be determined.

When examining Table 3.1., the two-year averages of the macro minerals found in the seeds of *Viburnum opulus* L., *Viburnum lantana* L., *Viburnum orientale Pallas*, and *Viburnum tinus* L. species were determined as follows: Sodium (Na) mineral, 294685,73 ppb, 264084,66 ppb, 449832,51 ppb, 331361,55 ppb, respectively; Potassium (K) mineral, 7507134,88 ppb, 5703430,77 ppb, 10978999,20 ppb, 14310484,83 ppb, respectively; Calcium (Ca) mineral, 1,097535,43 ppb, 885633,21 ppb, 1073943,12 ppb, 1569977,49 ppb, respectively; Magnesium (Mg) mineral, 2876076,10 ppb, 1917113,06 ppb, 2645858,14 ppb, 2914929,58 ppb, respectively; and Phosphorus (P) mineral, 3083021,91 ppb, 2069287,35 ppb, 3662347,54 ppb, 2641958,80 ppb, respectively. In addition to these macro minerals, Boron (B), Aluminum (Al), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Rubidium (Rb), Strontium (Sr), Tin (Sn), and Barium (Ba) minerals were also detected at certain levels in the seeds of all species. Among the macro minerals in the seeds, *Viburnum orientale Pallas* was determined to be the richest species in terms of Na and P, while *Viburnum tinus* was found to be the richest species in terms of Mg, K, and Ca. The ash content of the seeds was also found to be consistent with the mineral content, with *Viburnum tinus* L. and *Viburnum orientale Pallas* being the species with the highest ash content.

When examining the mineral content of *Viburnum* species, it was found that there are no mineral analyses of seeds in the literature, but analyses have been conducted on fruits. Kalyoncu et al. (2013) showed that the fruits of *Viburnum opulus* are rich in K, P, Ca, and Mg (10764,764 ppm, 1304,169 ppm, 1228,711 ppm, 1289,088 ppm, respectively). Akbulut et al. (2008) reported that the two highest minerals among 18 minerals were K and Ca (8420,00 ppm, 2441,00 ppm, respectively). Taşkin et al. (2019) showed that except for the copper mineral, the amounts of other minerals in the leaves of *Viburnum opulus* were higher than in its fruits and stems.

CONCLUSION

Based on these results, it has been determined that the identified physicochemical properties of *Viburnum* species can vary depending on the species and the harvest years. The fruits of *Viburnum* L. species are particularly rich in dietary fiber, and although the seeds of *Viburnum* L. species have low fat content, they are quite rich in protein, dietary fiber, and minerals, indicating that they possess functional properties. The results obtained from this study contribute to the literature on the species of the *Viburnum* genus growing in Turkey, benefiting many different scientific disciplines (biology, chemistry, food science, pharmacology, etc.).

Explanation

This full text has been prepared by the authors from a part of the ongoing doctoral thesis study of Benan DİNÇ GİRGİN.

ACKNOWLEDGMENT

The identification of the species of four different *Viburnum* L. samples used in this study in Turkey was carried out by Associate Professor Dr. Aslı KOCA from Hacettepe University, Faculty of Science, Department of Biology, Botany Division. I would like to express my gratitude to Associate Professor Dr. Aslı KOCA for her contributions to the study.

REFERENCES

Akbulut, M., Calisir, S., Marakoglu, T., Coklar, H. 2008. Chemical and technological properties of European cranberrybush (*Viburnum opulus L.*) fruits. Asian Journal of Chemistry, 20(3), 1875.

Aksoy, U. 1991. Kuru İncir Üretiminde Kaliteyi Etkileyen Faktörler. İncirde Verimlilik Paneli MPM, Aydın.

Alizadeh, N. S. H. H., Öztürk, R., Acar, A. İ. 2007. Some physical properties of gilaburu seed. Tarim Bilimleri Dergisi, 13(3), 308-311.

Altun, M. L., Özbek, H., Çitoğlu, G. S., Yilmaz, B. S., Bayram, I., Cengiz, N. 2010. Hepatoprotective and hypoglycemic activities of Viburnum opulus L. Turk J Pharm Sci, 7, 35-48.

AOAC. 1985. Total dietary fibre in foods. Official methods of analysis of AOAC, 14th edition, Washington, DC.

Baytop, T. 1999. Therapy with medicinal plants in Turkey. Past and Present, 2, 348-349.

Cam, M., Hisil, Y., Kuscu, A. 2007. Organic acid, phenolic content, and antioxidant capacity of fruit flesh and seed of Viburnum opulus. Chemistry of Natural Compounds, 43(4).

Cemeroğlu, B. 2007. Gıda Analizleri, Gıda Teknolojisi Derneği Yayınları No: 34. Bizim Büro Basımevi.

Cemeroğlu, B. 2010. Gıda Analizleri, Gıda Teknolojileri Derneği Yayınları, No: 34. s 13-41-102-225.

Davis, P. H. 1970. Flora of Turkey and the East Aegean Islands. Vol. 3.

Dinç, M., Aslan, D., İçyer, N. C., Çam, M. 2012. Gilaburu suyunun mikroenkapsülasyonu. Gıda Teknolojileri Elektronik Dergisi, (7), 2, 1-11.

Elagamey, A. A., Abdel-Wahab, M., Shimaa, M. M. E., Abdel-Mogib, M. 2013. Comparative study of morphological characteristics and chemical constituents for seeds of some grape table varieties. Journal of American Science, 9(1), 447-454.

Ferrentino, G., Giampiccolo, S., Morozova, K., Haman, N., Spilimbergo, S., Scampicchio, M. 2020. Supercritical fluid extraction of oils from apple seeds: Process optimization, chemical characterization and comparison with a conventional solvent extraction. Innovative Food Science & Emerging Technologies, 64, 102428.

Herrera, C. M. 1987. Vertebrate-dispersed plants of the Iberian Peninsula: a study of fruit characteristics. Ecological monographs, 57(4), 305-331.

Kacar, B. 1994. Bitki ve toprağın kimyasal analizleri. Ankara Üniversitesi Ziraat Fakültesi Eğitim, Araştırma ve Geliştirme Vakfı.

Kaliniewicz, Z., Choszcz, D. J. 2021. Analysis of the physical properties of seeds of selected Viburnum species for the needs of seed sorting operations. Processes, 9(4), 711. **https://doi.org/10.3390/pr9040711**

Kalyoncu, İ. H., Ersoy, N., Elidemir, A. Y., Karalı, M. E. 2013. Some physico-chemical characteristics and mineral contents of gilaburu (*Viburnum opulus L.*) fruits in Turkey. International Journal of Agricultural and Biosystems Engineering, 7(6), 424-426.

Kamel, B. S., Dawson, H., Kakuda, Y. 1985. Characteristics and composition of melon and grape seed oils and cakes. Journal of the American Oil Chemists' Society, 62(5), 881-883. **https://doi.org/10.1007/BF02541750**

Keskin, K. Ö. M. G. N., Kaya, T. 2011. Some Physical and Chemical Characteristics of Gilaburu (*Viburnum opulus L.*) Fruits in Erzincan Region. Iğdır Univ. J. Inst. Sci. & Tech. 1(4): 9-14.

Konarska, A., Domaciuk, M. 2018. Differences in the fruit structure and the location and content of bioactive substances in Viburnum opulus and Viburnum lantana fruits. Protoplasma, 255, 25-41.

Levent Altun, M., Saltan Çitoğlu, G., Sever Yilmaz, B., Çoban, T. 2008. Antioxidant properties of Viburnum opulus and Viburnum lantana growing in Turkey. International Journal of Food Sciences and Nutrition, 59(3), 175-180. https://doi.org/10.1080/09637480701381648

Minitab. 2021. Minitab Statistical Software, Minitab, Inc., State College, Pennsylvania.

Naumann, C., Bassler, R., Seibold, R., Barth, C. 1983. Chemiche Untersuchung von Futtermitteln Verlag. J. Neumann Neudamm. 10 (6). W. Germany.

Ozrenk, K., Ilhan, G., Sagbas, H. I., Karatas, N., Ercisli, S., Colak, A. M. 2020. Characterization of European cranberrybush (Viburnum opulus L.) genetic resources in Turkey. Scientia Horticulturae, 273, 109611.

Özbilgin, S., Ergene, B., Altun, M. L., Yılmaz, B. S., Saltan, G., Yüksel, E. 2015. HPLC method for the analysis of chlorogenic acid of Viburnum tinus L. and Viburnum orientale Pallas. Turkish Journal of Pharmaceutical Sciences, 12(2), 130-136.

Polka, D., Podsędek, A., Koziołkiewicz, M. 2019. Comparison of chemical composition and antioxidant capacity of fruit, flower and bark of Viburnum opulus. Plant Foods for Human Nutrition, 74, 436-442. https://doi.org/10.1007/s11130-019-00759-1

Ran, H., Liu, Y., Wu, C., Cao, Y. 2020. Phylogenetic and comparative analyses of complete chloroplast genomes of Chinese Viburnum and Sambucus (*Adoxaceae*). Plants, 9(9), 1143. **https://doi.org/10.3390/plants9091143**

Skujins, S. 1998. Handbook for ICP-AES (Varian-Vista). A Short Guide to Vista Series ICP-AES Operation (Version 1.0). Varian International AG, Zug, Switzerland.

Taşkın, O., AŞIK, B. B., Nazmi, İ. Z. L. İ. 2019. Gilaburu bitkisinin (*viburnum opulus l.*) meyve, sap ve yaprağının mineral içeriği. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 22(2), 178-182.

Wang, X. Y., Shi, H. M., Li, X. B. 2010. Chemical constituents of plants from the genus Viburnum. Chemistry & Biodiversity, 7(3), 567-593.

Xiang, C. L., Dong, H. J., Landrein, S., Zhao, F., Yu, W. B., Soltis, D. E., Peng, H. 2020. Revisiting the phylogeny of Dipsacales: new insights from phylogenomic analyses of complete plastomic sequences. Journal of Systematics and Evolution, 58(2), 103-117.

Yan, X., Liang, S., Peng, T., Zhang, G., Zeng, Z., Yu, P., Deng, S. 2020. Influence of phenolic compounds on physicochemical and functional properties of protein isolate from Cinnamomum camphora seed kernel. Food Hydrocolloids, 102, 105612.
https://doi.org/10.1016/j.foodhyd.2019.105612

Yeniçeri, Ş. A., Küçüköner, E. 2020. Zivzik Narı Çekirdek Yağının Yağ Asidi Kompozisyonun Belirlenmesi. Avrupa Bilim ve Teknoloji Dergisi, (19), 821-826.

Yılmaz, N., Yaylı, N., Mısır, G., Çoskunçelebi, K., Karaoğlu, Ş. A., Yaylı, N. 2008. Chemical composition and antimicrobial activities of the essential oils of Viburnum opulus, Viburnum lantana and Viburnum orientala.

Yunusova, S. G., Karimova, A. R., Tsyrlina, E. M., Yunusov, M. S., Denisenko, O. N. 2004. Change on storage of biological activity of Viburnum opulus seed components. Chemistry of natural compounds, 40, 423-426.

Yürüker, A., Çalis, I., Baser, K. H. C., Özek, T. 1995. Composition of the Essential Oil from Viburnum orientale Pallas Leaves. Journal of Essential Oil Research, 7(3), 321-323. https://doi.org/10.1080/10412905.1995.9698528

EDIBLE INSECTS AS ALTERNATIVE PROTEIN SOURCES

İsa EKİN[1], Nilgün ERTAŞ[1]

[1] Necmettin Erbakan University, Engineering Faculty, Food Engineering Department, Konya, Turkey
Corresponding author e-mail: isaekin41@hotmail.com

ABSTRACT

With the increase in the world's population, possible deficiencies in protein sources have come to the fore. Inadequacies in protein sources will also bring nutritional deficiencies. With the limited natural resources, the fact that accessible, affordable, nutritious, healthy, reliable and sustainable food production will become even more important in the future emerges. For this purpose, insects, seaweed, genetically modified organisms and artificial meat are thought to be used as food or food components needed as a protein source in the future. Although reasons such as not being accepted by the society and ethical concerns are among the factors preventing the commercialization of artificial meat, it is thought that artificial meat produced in a controlled manner can be presented as a healthier and safer production by reducing the risk of disease originating from raw materials. Algae, which are rich in protein, essential amino acids and vitamins, are also considered to be among the alternative food sources with their biomass and rich biodiversity. In addition, edible insects provide the opportunity for high-volume production in smaller areas with lower feeding costs and less waste compared to traditional meat production. In this context, the nutritional properties of edible insects, which are seen as alternative food or food ingredients and contain high amounts of bioactive substances as well as high protein content, are examined in this compilation study, focusing on their production and use as food and their effects on the environment and human health.

Keywords: Nutritional deficiencies, protein sources, edible insects, alternative foods.

INTRODUCTION

One of the concerns that has emerged today is the sustainable nutrition of people, which is becoming more important with the increase in the world's population. In order to feed the world population, which will reach approximately 9.1 billion people in the near future, the amount of food produced needs to be doubled on average (FAO, 2022). Considering that even today, 1.3 billion people do not have access to food; more than 2 billion people are affected by micronutrient deficiencies and cannot be nourished adequately, it is predicted that serious difficulties will be experienced in meeting the food requirement at an adequate level in the future and that production will need to be increased or new alternative sources will need to be found to feed the growing population (FAO, 2019; WHO, 2021). It has become necessary to develop new policies to prevent food scarcity caused by factors such as increasing environmental pollution, changing climates, increased migration from rural to urban areas, decreases in agriculture and animal husbandry, and food waste (Özertan, 2020). However, considering the limited resources, the carbon and water footprint of overproduction, and the limitations that global warming will bring, it becomes clear that new policies should be addressed from a different perspective (Andaç and Yılmaz Tuncel, 2023). In this respect, insect, microalgae and bacterial proteins that have high nutritional properties and environmentally friendly breeding conditions can be shown as alternatives to traditional protein sources (Van der Spiegel et al., 2013). Due to their high nutritional values and environmentally friendly breeding conditions, the consumption of edible insects as an alternative food source is recommended by the Food and Agriculture Organization of the United Nations (FAO, 2013; Aksoy and El, 2021). The ease of accessibility, high fertility rates, fast growth, being an economic source and being environmentally friendly play an important role in the acceptance of edible insects as sustainable food (Halloran et al., 2018). When the production of 1 unit of protein produced from beef, pork or chicken meat is compared with the production of the same amount of insect protein; It has been determined that with more cultivation area, more water and feed resource usage, greenhouse gas production is also quite high (Gahukar, 2016).

Although the concept of "entomophagy", which is not a new phenomenon for humans and means consuming insects as food, has a very old history, it has attracted considerable attention in recent years (Evans et al., 2015; Selçuk and Gencal, 2023). Archaeological findings prove that insects have a deep-rooted history throughout the evolutionary history of humans (Peniche, 2021). Although there are consumers who have a negative attitude towards edible insects, when the nutritional and culinary cultures of countries are examined, it is revealed that they can change over the years and that many products that are not consumed as food can become traditional foods. For example, frog meat consumption, which was previously only in the diet of the French, has become popular worldwide; lobster, which was considered worthless, was added to the diets of servants and prisoners as a punishment, but today it has begun to be defined as a food with high social value (Bukkens, 2005; Tao and Li, 2018). In addition, 'sushi', which was unknown in European countries until recently, has started to be consumed in many countries, including our country. These examples are an indication that cultures, habits and negative attitudes towards new foods may change in the future (Baş Aksoy and El, 2021).

With over one million known species, insects are the group with the most species diversity among animals. Interest in edible insects and the number of scientific publications on this subject have been increasing every year, especially since the 2010s (Del Mastro, 2021). Although the diversity of edible insects varies by region, the most frequently consumed insects are reported to be crickets, ants, mealworms, locusts, butterflies, moths, cockroaches and flies (FAO, 2013). The species and numbers of edible insects recorded in the world are given in Table 1.

Table 1. Edible Insect Species and Numbers

Class	Common Names	Number of species
Thysanura	Silverfish	1
Anoplura	Lice	3
Ephemeroptera	Mayflies	19
Odonata	Dragonfly	29
Orthoptera	Grasshopper, Cockroach, Cricket	267
Isoptera	Termites	61
Hemiptera	Half Wings	102
Homoptera	Cicadas, Dwarf Cicadas, Cotton Lice	78
Neuroptera	Nerve Wings	5
Lepidoptera	Butterflies, Moths, Silkworms, Caterpillars	253
Trichoptera	Crownflies	10
Diptera	Flies, Mosquitoes	34
Coleoptera	Beetles	468
Hymenoptera	Ants, Bees, Wasps	351
	Total	1.681

Ramos and Elorduy, (2005)

Although insects are common food in many parts of the world, they are viewed with suspicion by many Western consumers. However, it is believed that in the near future they will think otherwise. The main reason for turning edible insects into a normal food ingredient today is that they are delicious, nutritious, sustainable and healthy (Del Mastro, 2021).

Nutritional Properties of Edible Insects

Since the number of edible insect species exceeds 2000, the types and amounts of nutrients differ among species (Rumpold and Schlüter, 2013). Insects are rich in proteins, fats and dietary fibers and provide approximately 400-500 kcal of energy per 100 g depending on the species (Rumold and Schlüter, 2015). Insects have a protein content of 38% to 77% of their total dry weight. Many insect species have been reported to have higher protein content per 100 g dry weight (e.g. crickets 68.7%) than red meat (27.4%) or cod (28.5%) (Gahukar, 2011). A comparison of some edible insects in terms of nutritional values is given in Table 2.

Table 2. Comparison of some edible insects in terms of nutritional values

Nutrients	Cricket	Butterfly	Mealworm	Cockroach	Grasshopper	Fly	Ant
Moisture (%)	69.2	73.9	63.7	71.2	62.7	3.8	74
Ash (%)	3.6	3.7	3.3	4.3	3.2	8.6	6.5
Protein (%)	66.6	45.6	65.3	78.8	71.8	47	63.5
Oil (%)	22.1	35	14.9	20	10.2	32.6	13.5
Dietary fiber (%)	10.2	6.5	20.4	-	6.4	6.7	6.9

Baş Aksoy and El, 2021;USDA National Nutrient Database, 2015; Bukkens, 1997; Finke, 2007, 2013; Ramos-Elorduy and ark., 1997. Cırcır böceği (Acheta domesticus),Kelebek (Anaphe panda, larva), Un kurdu (Tenebrio molitor, yetişkin), Hamam böceği (Blatella germanica), Çekirge (Zonocerus sp.), Sinek (Hermetiaillucens, larva), Karınca (OIecophylla smaragdina).

Since insect proteins are highly digestible, consumption of insects can significantly increase the nutritional quality of human nutrition by contributing to total protein intake (Bukkens, 1997; Belluco et al., 2013). Almost all edible insect species meet the essential amino acid content (e.g. phenylalanine, tyrosine, tryptophan, threonine and lysine) recommended by the World Health Organization (2007) (WHO, 2007). The total fat content of insects varies between 2% and 62% (Williams et al., 2016). In terrestrial trials, edible insects have been reported to contain higher amounts of long-chain polyunsaturated fatty acids, especially omega-6 fatty acids, than aquatic insects (Fontaneto et al., 2011). The mineral content of edible insects also varies greatly among species. Insects are generally low in calcium and potassium. Crickets and grasshoppers are rich in magnesium; cricket powder has been found to contain high levels of magnesium, copper and zinc (Rumpold and Schlüter, 2013; Montowska et al., 2019). Although information on the vitamin content of edible insects is limited, it has been reported that some species contain relatively high levels of B complex vitamins (riboflavin, pantothenic acid and biotin) and have a low vitamin C content (Rumpold and Schlüter, 2013; Finke and Oonincx, 2014; Klaus and Yukiko, 2021). The exoskeleton of insects, which contains a significant amount of fiber, consists of chitin, a long-chain N-acetyl-glucosamine polymer that constitutes approximately 10% of the dry weight (Belluco et al., 2013; Van Huis et al., 2013).

It has been reported that the addition of edible insects to diets may have positive effects on health by increasing the gastrointestinal system and human immunity as well as reducing the risk of bacterial infections (Nowakawski, Miller, Xiao, & Wu, 2021). Chitosan, a form of chitin in particular, is considered as a functional food ingredient that provides potentially beneficial effects on wound healing, colonic and cardiovascular health, cholesterol reduction, and innate and adaptive immune responses (Prosky, 2000; Shahidi & Abuzaytoun, 2005; Lee et al., 2008; Tripathi & Singh, 2018; Klaus & Yukiko, 2021).

Production, Consumption and Environmental Impact of Edible Insects

Today, 92% of the insects consumed are traditionally collected from nature, while the rest are obtained as a result of industrial production (Demirci and Yetim, 2021). Different types of insects are collected from nature in regions with high temperatures. However, this sometimes causes the ecological balance to be disrupted. It has been reported that with the increase in edible insect consumption in the future, cultivation conditions should be provided under the name of mini-animal husbandry (Ramos Elorduy, 2006; Akpalu et al., 2009; Van Huis, 2015; Selçuk and Gencal, 2023). With the recommendation of the Food and Agriculture Organization of the United Nations (FAO) for the consumption of edible insects, many companies have established insect farms and started to raise insects under hygienic conditions (FAO, 2013). In addition, insects are ground into powder and put on the market under names such as insect powder or insect flour, and many companies producing insect-based foods have started to produce this product, which is in the form of flour, by adding it to foods (Van Huis, 2013). Since insects do not require advanced technology for production, they are thought to be a potential food production source for poor and technologically underdeveloped countries (Kibar, 2017).

In Thailand, where there are twenty thousand farms, it has been reported that 7,500 tons of insects are produced annually (Hanboonsong et al., 2013). Although in the West, they are mostly grown for pet food, some companies in the Netherlands also grow for human consumption (Van Huis, 2015). In Turkey, the only insect production farm in our country, established in Antalya for the sole purpose of producing animal feed, sometimes also meets the demands for human consumption (Selçuk and Gencal, 2023).

The market size of edible insects is estimated to be approximately 8 billion dollars by 2030 (Van Huis, Rumpold, Van Der Fels-Klerx, & Tomberlin, 2021). The export and import of edible insects in Southeast Asia and Africa is of great economic importance (Tanga et al., 2021). Although there are more than 1 million insect species worldwide, the insects that are particularly commercialized for human consumption are locusts and mealworms (FAO, 2021; Ponca-Reyes & Lessard, 2021; Andaç & Yılmaz Tuncel, 2023).

In Switzerland, where insects are considered food, insect-based hamburgers have become one of the accepted foods in the country as of 2017. In countries such as the USA and Canada, crackers and snacks made with insect flour are offered on the market (Van Huis, 2013). In Western countries, where insect consumption is associated with unhygienic or spoiled food; insects have never gained an important place in food cultures and are therefore not yet accepted as a source of protein (Megido et al., 2016). When insects are considered as food, two main different reactions occur in people. In countries where insects are traditionally consumed, insects are considered a valuable source of protein, while in Western cultures, insects are considered dirty, disgusting and dangerous (Jensen and Lieberoth, 2019). However, it has been reported that individuals are willing to change their thoughts about entomophagy if they are informed about its health and environmental benefits (Mancini et al., 2019).

Compared to traditional farm animals and poultry, insects support sustainability in terms of nutrition and environmental health due to reasons such as less greenhouse gas generation and less land and energy use (Muslu, 2020). Insects consume much less water compared to other animals. Production can be done in much smaller areas compared to the land used for raising farm animals (Smil, 2002; Ramos-Elorduy, 2008; Nelson et al., 2009; FAO, 2013; Van Huis, 2013; Gahukar, 2016; Halloran et al., 2016; Baş Aksoy and El, 2021). In addition, insects grow much more easily and in a shorter time than large animals and have a high fertility rate, which is important in terms of being a sustainable protein source. When the production of these two sources is compared, it has been reported that 2 kg of feed is spent to produce 1 kg of edible insects, while 25 kg of feed is spent to produce 1 kg of beef (Guine et al., 2021).

Since the consumption of insects as food causes concerns in terms of food safety, regulations should be made regarding the health effects of insects and their products, like other food products, and production hygiene (Van Huis et al., 2013). Microbiological, parasitological, chemical, toxicological and allergenic risks should be emphasized (Gahukar, 2011). Due to the high consumption of butterfly larvae, in a toxicological study conducted in Nigeria, untreated larvae showed toxic effects when given to mice. However, the possibility of neurotoxin effects was eliminated by boiling and drying the larvae in the sun (Akinnawo et al., 2002). Microbial risks associated with edible insects and their products can be significantly controlled by good hygiene practices to be applied during cultivation, harvesting, processing, storage and transportation (FAO, 2021). However, spores of pathogenic microorganisms, especially some genera such as Bacillus, may occur during production and cultivation (Osimani and Aquilanti, 2021). In addition, although the risks of foodborne viruses posed by the consumption of edible insects are limited, attention should be paid to the risk of virus contamination through feed. In addition, there are yeasts (Candida, etc.) and molds (Aspergillus, Alternaria, etc.) associated with edible insects (FAO, 2021).

The lack of a legal regulation that would allow the use of insects as food and feed is seen as the biggest obstacle to the industrial growth of this sector in developed countries. It is thought that the concept of “novel food” could pave the way for the use of insects as human food (Van Huis et al., 2013). Although some legal regulations and control mechanisms have been established regarding the hygiene and food safety of edible insects in countries such as the European Union, the United States of America and Canada, regulations on the subject are very rare or non-existent in continents such as Africa and Asia and in Mexico (Raheem et al., 2019; FAO, 2021; Andaç and Yılmaz Tuncel, 2023).

CONCLUSION

Today a significant portion of the animal protein demand worldwide is met through the traditional production of cattle, pigs and poultry. It does not seem possible to provide sufficient production due to the future population and demand growth of traditional production methods. In addition, it is understood that the negative results such as greenhouse gas emissions and high land and water usage caused by these low-yield traditional production methods may lead to major problems in the future. Adding edible insects and their products, which are environmentally friendly protein sources, to the diet is important in terms of meeting the increasing protein demand. However, insects will provide an alternative to animal proteins when produced on an industrial scale. For this purpose, it is necessary to increase the necessary research and development studies, to switch to industrial scale production, to eliminate food safety concerns and to make legal arrangements to provide a certain control mechanism. However, it should be taken into account that there are cultural differences regarding the consumption of edible insects in different regions.

REFERENCES

Andaç, A.E., Yılmaz Tuncel, N. 2023. Sürdürülebilir ve Yeni Bir "Gıda" Alternatifi Olarak Yenilebilir Böcekler. Çanakkale Onsekiz Mart University Journal of Advanced Research in Natural and Applied Sciences, Cilt 9, Sayı 1, Sayfa: 251-267.

Akinnawo OO, Abatan MO, Ketiku AO. 2002. Toxicological study on the edible larva of Cirina forda (Westwood). African Journal of Biomedical Research, 5: 43-46.

Baş Aksoy ve El, 2021. Protein Source of the Future: Edible Insects. Turkish Journal of Agriculture-Food Science and Technology, 9(5): 887-896, 2021.

Bessa, L. W., Pieterse, E., Sigge, G. ve Hoffman, L. C. 2020. Insects as human food; from farm to fork. Journal of the Science of Food and Agriculture, 100(14), 5017-5022.

Belluco, S., Losasso, C., Maggioletti, M., Alonzi, C.C., Paoletti, M.G., and Ricci, A. 2013. Edible insects in a food safety and nutritional perspective: a critical review. Compr. Rev. Food Sci. Food Saf. 12(3): 296-313

Bukkens, S.G. 1997. The nutritional value of edible insects. Ecol. Food Nutr. 36(2-4): 287-319.

Bukkens SGF. 2005. Insects in the Human Diet: Nutritional Aspects. In: Paoletti MG (Editörler), Ecological Implicationsof Minilivestock: Potential of Insects, Rodents, Frogs, and Snails. Science Publishers, Enfield, NH, pp. 545-577. ISBN 1-57808-339-7 (Online).

Del Mastro, N. L. 2021. Evolution of the Interest on Edible Insects. American Journal of Biological and Environmental Statistics, 7(2), 52-56.

Demirci M., Yetim H. 2021. İnsan gıdası olarak böcek proteinleri tüketimi ve getirdiği sorunlar. Helal ve Etik Araşt. Derg, 3(2): 11-22.

FAO, 2013. Edible insects. Future prospects for food and feed security Food and Agriculture Organization of the United Nations Rome.

FAO, 2019. Food and Agriculture Organization of the United Nations World Health Organization, Sustainable Healthy Diets Guiding Principles, Food and Agriculture Organization of the United Nations Rome.

FAO, 2021. Looking at edible insects from a food safety perspective. challenges and opportunities for the sector. Rome.

FAO, 2022, How to feed the world in 2050., https://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf.

Finke MD. 2007. Estimate of chitin in raw whole insects. Zoo Biology 26: 105-115.

Gahukar, R.T. 2016. Chapter 4-Edible Insects Farming: Efficiency and Impact on Family Livelihood, Food Security, and Environment Compared With Livestock and Crops. In A. T. Dossey, J. A. Morales-Ramos ve M. G. Rojas (Editörler), Insects as Sustainable Food Ingredients, 85-111. San Diego: Academic Press.

Gahukar, R.T. 2011. Entomophagy and human food security. International Journal of Tropical Insect Science, 31: 129-144.

Gahukar, R.T. 2016. Edible Insects Farming: Efficiency and Impact on Family Livelihood, Food Security, and Environment Compared with Livestock and Crops, In: Dossey AT, Morales-Ramos JA, Guadalupe Rojas M (Editörler), Insects as Sustainable Food Ingredients: Production, Processing and Food Applications. pp. 85-106.

Guine RPF, Correia P, Coelho C, Costa CA. 2021. The role of edible insects to mitigate challenges for sustainability, Open Agriculture, 6: 24-36.

Hanboonsong, Y., Jamjanya, T., & Durst, P. B. 2013. Six-legged llvestock: Edible insect farming, collection and marketing İn Thailand. Food and Agriculture Organization of the United Nations, Regional Office for Asia and the Pacific.

Halloran A, Roos N, Eilenberg J, Cerutti A, Bruun S. 2016. Life cycle assessment of edible insects for food protein: A review. Agronomy for Sustainable Development, 36(57): 6-13.

Halloran, A., Flore, R., Vantomme, P. ve Roos, N. 2018. Edible Insects in Sustainable Food Systems. ISBN 978-3-319-74011-9 (eKitap).

Jensen, N. H., Lieberoth, A. 2019. We will eat disgusting foods together-Evidence of the normative basis of Western entomophagy-disgust from an insect tasting. Food Quality and Preference, 72, 109-115.

Kibar S. (2017). Böcek Yemenin Nesi Yanlış? Çankırı Karatekin Üniversitesi Sosyal Bilimler Enstitüsü Dergisi, 8(1): 96-113.

Lee, C.G., Da Silva, C.A., Lee, J.-Y., Hartl, D., ve Elias, J.A. 2008. Chitin regulation of immune responses: an old molecule with new roles. Curr. Opin. Immunol. 20(6): 684-689.

Megido RC, Gierts C, Blecker C, Brostaux Y, Haubruge E, Alabi T, Francis F. 2016. Consumer acceptance of insect-based alternative meat products in Western countries, Food Quality and Preference 52: 237-243.

Montowska, M., Kowalczewski, P.Ł., Rybicka, I., and Fornal, E. 2019. Nu-tritional value, protein and peptide composition of edible cricket powders. Food Chem. 289: 130-138.

Muslu M. 2020. Sağlığın Geliştirilmesi ve Sürdürülebilir Beslenme İçin Alternatif Bir Kaynak: Yenilebilir Böcekler. Gıda, 45(5): 1009-1018.

Nelson GC, Rosegrant MW, Koo J, Robertson R, Sulser T, Zhu T, Ringler C, Msangi S, Palazzo A, Batka M, Magalhaes M, Valmonte-Santos R, Ewing M, Leeve D. 2009. Climate Change: Impact on Agriculture and Costs of Adaptation. International Food Policy Research Institute. Washington, D.C.

Nowakowski, A. C., Miller, A. C., Miller, M. E., Xiao, H., Wu, X. 2021. Potential health benefits of edible insects. Critical Reviews in Food Science and Nutrition, 62(13), 3499-3508.

Osimani, A., Aquilanti, L. 2021. Spore-forming bacteria in insect-based foods. Current Opinion in Food Science, 37, 112-117.

Özertan, G. 2020. Sürdürülebilir Büyüme Bağlamında Tarım ve Gıda Sektörünün Analizi, TÜSİAD-T/2020-03/613.

Peniche, P.C.Y.C. 2021. Drivers of insect consumption across human populations. Evolutionary Anthropology.

Ponce-Reyes R., Lessard B. D. 2021. Edible Insects - A roadmap for the strategic growth of an emerging Australian industry. CSIRO, Canberra.

Prosky, L. 2000. When is dietary fiber considered a functional food? Bio-factors 12(1-4): 289-297.

Raheem, D., Raposo, A., Oluwole, O. B., Nieuwland, M., Saraiva, A., Carrascosa, C. 2019. Entomophagy:nutritional, ecological, safety and legislation aspects. Food Research International, 126, 108672.

Ramos-Elorduy J, Pino JMP, Prado EE, Perez MA, Otero JL, de Guevara OL. 1997. Nutritional Value of Edible Insects from the State of Oaxaca, Mexico, Journal of Food Composition and Analysis, 10: 142-157.

Ramos-Elorduy, J. 2005. Insects: A hopeful food source. Science Publications.

Ramos-Elorduy J. 2008. Energy Supplied by Edible Insects from Mexico and their Nutritional and Ecological Importance. Ecology of Food Nutrition, 47: 280-97.

Rumpold BA, Schlüter OK. 2013. Nutritional composition and safety aspects of edible insects. Molecular Nutrition and Food Research, 57: 802-823.

Selçuk, G.N., Gencal, A., 2023. Alternatif Gıda Olan Yenilebilir Böcekler. Atatürk Üniversitesi Gastro-Dünya Dergisi / Cilt 2 / Sayı 2.

Shahidi, F., ve Abuzaytoun, R. 2005. Chitin, chitosan, and co-products: chemistry, production, applications, and health effects. Adv. Food Nutr. Res. 49: 93-135.

Smil, V. 2002. Eating Meat: Evolution, Patterns, and Consequences. Population and Development Review, 28: 599-639.

Tanga, C. M., Egonyu, J. P., Beesigamukama, D., Niassy, S., Emily, K., Magara, H. J., Omuse, E. R., Subramanian S., Ekesi, S. 2021. Edible insect farming as an emerging and profitable enterprise in East Africa. Current Opinion in Insect Science, 48, 64-71.

Tao J, Li YO. 2018. Edible insects as a means to address global malnutrition andfood insecurity issues. Food Quality and Safety, 2: 17-26.

UN DESA, 2019. World population prospects 2019: highlights. United Nations Department for Economic and Social Affairs.

Van der Spiegel M, Noordam MY, Van der Fels-Klerx HJ. 2013. Safety of Novel Protein Sources (Insects, Microalgae, Seaweed, Duckweed and Rapeseed) and Legislative Aspects for Their Application in Food and Feed Production. Comprehensive Reviews in Food Science and Food Safety, 12(6): 662-678.

Van Huis, A., van Itterbeeck, J., Klunder, H., Mertens, E., Halloran, A., Muir, G., ve Vantomme, P. 2013. Edible insects: Future prospects for food and feed security. FAO Forestry Paper 171. Rome, Italy: Food and Agriculture Organization of the United Nations.

Van Huis, A. 2015. Edible insects contributing to food security, Agriculture and Food Security, 4(20), 1- 9.

Van Huis, A., Rumpold, B. A., Van Der Fels-Klerx, H. J., Tomberlin, J. K. 2021. Advancing edible insects as food and feed in a circular economy. Journal of Insects as Food and Feed, 7(5), 935-948.

World Health Organization. 2007. Protein and amino acid requirements in human nutrition: Report of a joint WHO/FAO/UNU expert consul-tation. WHO technical report series 935. World Health Organization, Geneva.

World Health Organization (WHO), United Nations Children's Fund (UNICEF), International Bank for Reconstruction and Development/The World Bank. 2021. Levels and trends in child malnutrition: key findings of the 2021 edition of the joint child malnutrition estimates.

NANOTECHNOLOGICAL APPLICATIONS in FOOD PACKAGING: IMPACT on FOOD SHELF LIFE

Hilal Soyocak , Nebahat Şule Üstün & Sadettin Turhan

Ondokuz Mayıs University / Department of Food Engineering

SUMMARY

Foods are packaged to protect them from various environmental influences such as heat, light, moisture, chemical and microbial contamination. Traditional packaging is a passive application that covers the food only to protect it from environmental influences. In this application, different materials are used to determine the most suitable packaging material for the food. Active packaging, which is a new approach in packaging, has been dveloped to extend shelf life of the food and to ensure minimum loss of food quality in this process. Nanotechnological applications, one of the latest strategies used in food packaging, is one of the active packaging methods. Nanotechnology is a field that deals with nano material with a size between 1-100 nm. Nanotechnologyical applications can be grouped as organic and inorganic according to their origin, as well as nanosheets, nanotubes and nanoparticle according to their size. Moisture and gas barrier properties and mechanical properties can be improved in edible films and coatings developed with nanotechnological applications. In addition, microbial and oxidative spoilage of foods can be prevened or delayed and thus the shelf life of foods can be extended. Nanoparticle added to the packaging material both improve the propeties of the food packaging material and ensure the sustained release of antioxidants and other active ingredients from the food surface. Metal nanoparticles such as silver, gold, titanium dioxide, magnessium oxide, zinc oxide are the most commonly used in packaging materials due to their antioxidant and antimicrobial properties. In this review, a broad summary of studies on the effect of nanotechnological processes applied to food packaging on the shelf life of various foods is presented.

Keywords: nanotechnology, nanoparticle, shelf life, active packaging

INTRODUCTION

Oxidation, which occurs during food storage and adversşy affects the shelf life, quality and nutritional value of the product, involves many complex chemical reactions. Various methods are used to eliminated thede negative effects and control oxidation. Elimination of pro-oxidants, addition of antioxidant agents, controlling storage conditions and using different packaging materials are examples of methods used to delay or prevent oxidation. Active packaging systems containing antioxidant agents to prevent food oxidations added directly to the product are more effective in preventing oxidation reactions that start on the food surface. Various nano materials are used to create antioxidant food packaging. These are nanoparticles, nano fibers, nano emulsions and nano crystals (Cheng et al., 2024). In foods packaged with edible films and coatings prepared with nanotechnological methods, even if the outer packaging material is opened, losses in properties such as nutrients, color and aroma of the food are reduced. The desired packaging property in food packaging is to have gas and moisture barrier properties an to be strong and biodegradable. Compounds such as bacteriocins and organic acids in packaging are obtained under diffucult conditions and have high sensitivity. Inorganic nanoparticles used in packaging Show strong antibacterial activity even at low concentrations and under extreme conditions. In the food industry, nanotechnology is used in food packaging to enhance packaging (improve physical properties), to create active packages (antioxidant, antimicrobial agents, etc.) and to create smart packages (pathogen detection, spoilage control, etc.). (Singh et al., 2017).

Active Packging in Food Packaging

We package food to protect it from various environmental influences such as heat, light, moisture, chemical and microbial contamination. Traditional packaging is a passive method of enclosing food to protect it from environmental factors. In these methods, different materials are used to determine the most suitable packaging material for the food. Avtive packaging, which is a new approach in packaging, has been developed to extend the shelf life of the food and to ensure minimum loss of food quality during this period. Active packaging systems are generally classified in two groups. These are active capture systems (absorbents) and active release systems (emitters) (Firous et al., 2021; Yıldırım et al., 2018).
Active packaging is defined by the European Comission as "the deliberate inclusion of ingredients that will release or absorb substances into the packaged food or the surrounding environment." Examples of ingredients included in the packaging material are antioxidants and antimicrobial agents. Traditionally, active ingredients are included in food. However, some disadvantages ocur in this case. For example, the ingredients added to the food have a negative sensory effect on the food or intraction between the added ingredient and the food components occurs, causeing the effect of the active ingredient to decrease. Active packaging aims to prevent such disadvantages. Some components used in active packaging can be listed as oxygen scavengers (iron, ascorbic acid, unsaturated hydrocarbon dienes, palladium, etc.), moisture scavengers, ethylene scavengers, antioxidants, carbon dioxide scavengers, antimicrobial agents and nanoparticle (Almasi et al., 2021; Firous et al., 2021; Yıldırım et al., 2018).
Different methods are used for active packaging of foods. (i) Placing pouches or pads containing the active ingredient in the package; this method is generally used for placing ingredients that are moisture retainers org as generators in the package. (ii) Coating the polymer surface with the active ingredient; this type of application is preferred in methods where heat sensitive components are used. The packaging material is coated with a thin layer of active ingredient. (iii) insertion of the active ingredient into the packaging polymer and the active ingredient. With the interaction between these functional groups, the active ingredients forms a strong bond with the polymer. However, there is a possibility of a decrease in the effect of the active ingredient in this method. (iv) polymers containing directly active ingredient; int his method, the active ingredients is added directly during the preparation of the polymer. Thus, the negative effect of the active ingredient is eliminated. Ths method is the most widely used method in active packaging. In packages prepared in this way, cotrolled release of the active ingredient is realized. All methods are schematized in Figure 1 (Almasi et al., 2021).

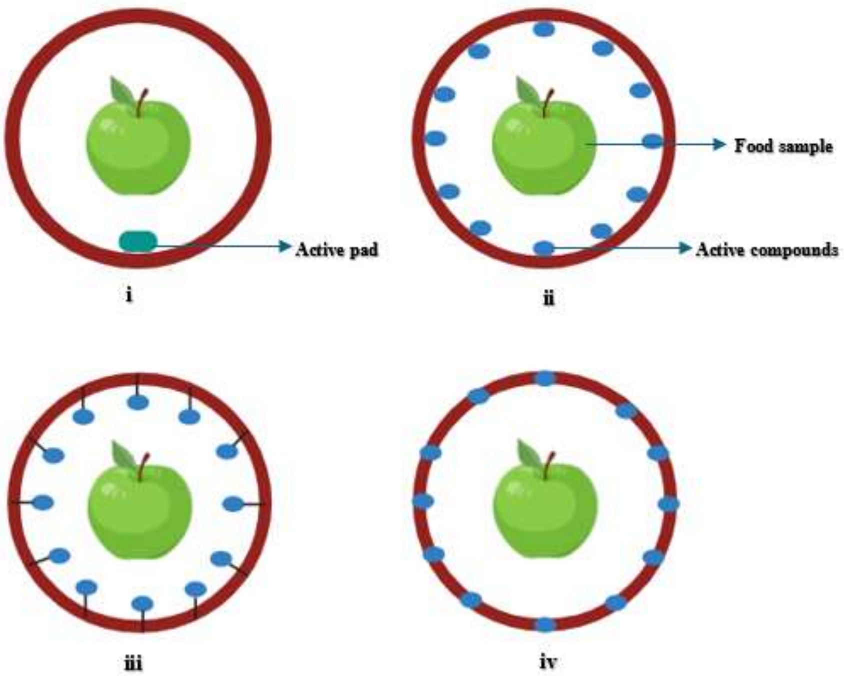


Figure 1. Active packaging methods

Nanotechnological Applications

Nanotechnological applications, one of the latest strategies used in food packaging, are gaining importance day by day. Nanotechnology is a field that deals with nano materials between 1-100 nm in size. Food packaging prepared with nanomaterials has many advantages compared to traditional methods. Nanoclays, one of the nanomaterials frequently studied by researchers, are preferred as food packaging due to their mechanical, thermal and barrier properties as well as their low cost. Nanoclays are composed of compounds such as montmorillonite, bentonite and kaolinite. Among these, montmorillonite and bentonite are recognized as GRAS by FDA (Almasi et al., 2021; He et al., 2019).
There are different classifications in nanotechnological applications. In the classsification accroding to their origin, they are divided into organic and inorganic, classification according to their size is in the form of nanosheets, nanotubes ans nanoparticle. With nano adible films and coatings developed with nanotechnological applications, it is desired to improve the moisture and gas barrier properties and mechanical properties of packaging. In addition, it is aimed to prevent or delay microbial and axidative spoilage of food and thus increase its shelf life. Nanotechnological applications are gaining importance especially fort he shelf life of fresh fruiys and vegetables that continue active respiration during transportation and storage efficiency in fruits and vegetables that continue active respiration. Studies of nano materials with features such as pesticide control, pathogen detection, toxin detection in active packaging with nanotechnology are carried out (Almasi et al., 2021; He et al, 2019).

The main reason for using nanotechnological applications is to improve the thermal, machanical and barrier properties of the developed packaging material. Active nanocomposite films are divide into two categories; (i) films containing nanostructured materials and (ii) films containing nanostructured materials as well as active components (Almasi et al., 2021):
Metal and metal oxide nanoparticles can be used in different applications in various fields. Nanoparticle synthesis can be categorized as physical, chemical and biological methods. Among biological synthesis methods, nanoparticle synthesis in plant extract media enables both the reduction of toxic by-products and the improvement of nanoparticle size tunng. Researchers believe that flavonoids in particular play an important role in the synthesis of many bioactive compounds found in plant extract media (Jeevanandam et al., 2016).
Biomedical and bioprocess applicaations are realized in the biological synthesis of nanoparticle. It has been determined that the toxic effect of metal nanoparticle obtained by this method is less. Three methods are used in the biological synthesis of nanoparticle. The first of these is the bacterial synthesis method. In this method, nanoparticle synthesis is carried out using different bacteria (Bacillus licheniformis, Pseudomonas meridiana, Lactobacillus casei, Shewanella alga, etc.). Researchers report that proteins, enzymes and other biochemicals play a role in nanoparticle synthesis in bacterial media. The biggest advantage of this method is that it allows the synthesis of large amounts of nanoparticles without the use of expensive and hazardous chemicals. However, it has the disadvantages of being time-consuming and not being able to control the properties of the nanoparticles such as size, shape and crystallinity. The second biological synthesis emthod is fungal synthesis. Fungi can be used as biological agents in the synthesis of metal and metal oxide nanoparticles thanks to their various intracellular enzymes. It has been determined that fungi have a higher ability to synthesize nanoparticle compared to bacteria. The advantages of nanoparticle synthesis by this method can be listed as having high metal retention capacity, producing high amounts of enzymes per biomass unit, and the growth of fungi on the surface of inorganic subsrates facilitates the dispersion of the metal. However, in addition to these advantages, the fact that it is time-consuming (fungal growth takes 24-120 hours) can be said to be a disadvantage compared to other methods. Nanoparticle synthesis in plant environment is the third group of biological methods. Plant biodiversity offers exciting biochemical and yield-specific resources for nanoparticle synthesis. Apart from bacteria and fungi, plant extracts are an important sources for nanoparticle synthesis. Studies on plant extract media have revealed the presence of many bioactive compounds such as alkaloids, amino acids, chelating proteins and these compounds enable the redustion of metal ions to nanoparticles. Nanoparticle synthesis from plant extracts takes under specific conditions. Temperature, pH and salt concentration are the main factors affectingnanoparticle synthesis (Jeevanandam et al., 2016).
Plant leaf extract are widely available, safe to use and have various metabolites that act as reducing agents in nanoparticle synthesis. The natüre of the plant leaf extract such as phytochemical concentration, metal salt concentration, pH, temperature and contact time are known to affect the nanoparticle production rate, production quantity, stability and quality. The main phytochemicals present in plant extract and responsible fort he synthesis of nanoparticle have been identified as terpenoids, flavones, ketones, aldehydes, amides and carboxylic acids (Jeevanandam et al., 2016).

Flavanoids are in the group of polyphenolic compounds and include compounds such as anthocyanins, isoflavonoids, flavonols. These compounds have the ability to actively chelate metal ions and reduce them to nanoparticles. Researchers predict that the flavonoid content in plant extracts serve as an important factor for the formation of metal oxide nanoparticles. Flavonoids stabilite the nanoparticles anda re important for reducing the toxicity of the nano-formulation. Since flavonoids possess antioxidant properties, the formation of reactive oxygen species produced by metal oxides can be reduced (Jeevanandam et al., 2016).

Nanotechnology has a wide range of applications in various fields of science and technology. Many nanoparticles attract the attention of various fields with their antimicrobial effect. Studies have shown that nanoparticles such as silver, silver oxide, titanium dioxide, silicon, copper oxide, zinc oxide, gold, calcium oxide and magnessium oxide have antimicrobial activity. For example, it has been determined that small nanoparticle have higher antimicrobial activity. Calcium oxide nanoparticle has a strong antimicrobial effect with its active oxygen and basicity (Dizaj et al., 2014).

Nanofibers, another nanotechnological method used in food packaging are linear materials with diameters less than 1000 nm and high length/diameter ratio. They can be prepared by methods such as high shear machanics, deep eutectic solvent and electrospinning. Among the nanofiber preparation methods, electrospinning is gaining importance due to its easy, continuous production and operation at room temperatures. Both natural polymers (gelatin, ethyl cellulose, starch, etc.) and chemically synthesized polymers (polyvinyl alcohol, policapro lactone, etc.) are suitable for nanofiber preparation by electrospinning (Cheng et al., 2024).

Effect of Nanıtechnological Applications on Shelf Life of Foods

Today, the interest in active packaging is increasing day by day in studies on the shelf life of foods. Active packaging plays an important role in maintaining the quality charecteristics of food, especially by protecting the food from environmental conditions. Bioactive packaging is being developed to protect various foods (vegetables, fruits, meat, fish, cheese, etc.) from both chemical and microbial spoilage. For this purpose, various herbal extract, essential oils were mostly used, but recently nanoparticles have also been frequently studied as preservatives (Hossen et al., 2022). In a study conducted bu Hosseini et al. (2022), they used cinnamaldehyde-coated chitosan nanoparticles in the packaging of fresh rainbow trout. At the end of the study, it was determined that trout coated with active packaging slowed down lipid oxidation and microbial growth. Zinc oxide nanoparticles (ZnO-NPs) and bamboo leaf extract were added to a chitosan-based film and it was determined that the prepared films have potential for use in food packaging. Bamboo leaf extract and ZnO-NPs showed synergistic effect and showed antibacterial properties especially against E.coli and S. aureus. It was stated that the active packaging prepared at the end of the study has the potential to increase the shekf life of foods (Liu et al., 2021). Iron oxide (FeO) and zinc oxide (ZnO) nanoparticles synthesized with clove extract were used in films prepared to increase the storage time of chicken meat. Films containing FeO-NPs had strong oxygen capture activity, while films containng ZnO-NPs were found to have high antimicrobial properties. Films containing nanoparticle improved the microbial, chemical and color properties of chicken meat stored at 4°C (Chandrasekar et al., 2024). Jamróz et al. (2019a) prepared an active package containing furcellaran, gelatin and Se-

AgNPs and applied it to kiwi samples bu dipping method. Samples coated with films containing 10% and 15% nanoparticles were compared with control and LDPE coated samples. At the end of 8 days, the control samples deteriorated more quickly than the coated samples containing nanoparticle. The elasticity and elongation at break values increased when the ratio of the added nanoparticle in the film increased. Nanoparticle added samples showed strong antibacterial effect against *S. aureus* and *E. coli*. A study was conducted to characterize furcellaran-based active composite films containig selenium nanoparticles, green tea and mate tea, and to investigate their antioxidant and antimicrobial effects. The antimicrobial activity of Se-NPs added films against *S. aureus* and *E. coli* was determined and the added green tea extract improved the antioxidant properties of the films. Films prepared as smart films were used to detect spoilage in fish. The changes occuring in the films placed in the packaging during storage were examined and it was determined that they have potential to be used as a smart label. After 72 hours of storage, a change in film color occured (Jamróz et al., 2019b). A study was conducted to investigate the effect of Se-NPs synthesized in nano turmeric (NC) environment against fish pathogenes (Aeromonas hydrophila, E. coli, Salmonella Thyhimurium, S. aureus) and NC/Se-NPs were found to have an effective inhibitory power against microorganism. Ampicillin and NC/Se-NPs formed a zone diameter of 24.3 and 24.6 mm against E.coli, respectively. When other microorganism were also examined, NC/Se-NPs showed higher zone diameter than the antibiotic. There was no significant difference between the minimum inhibition concentrations in E. coli ans S. Thphimurium (Gad et al., 2022).

Se-NPs were added to hazelnut and walnut packages and oxidative degradation was examined during 42 days of storage. When both samples were compared with the control groups, malondialdehyde (MDA) formation was less at the end of storage. In the same way, potato chips, pork, chicken and ready-to-eat meat meals were applied to the packaging material and MDA formation in each sample was less in Se-NPs added samples. It was determined that MDA formation was reduced bu 25%in these samples (Vera et al., 2018). Cid-Lopez et al. (2021) prepared coating solutions containing calcium oxide nanoparticle (CaO-NPs) and applied them to cucumbers on the day of harvest. It was determined that coatings containing nanoparticles has a positive effect on the appearance, pigment and antioxidant capacity of the sample. It was determined that the shelf life of cucumbers was extended by 24 days after harvest. In a study where seeds were treated with CaO-NPs, it was determined that it had a positive effect on seed growth (Ganhdi et al., 2021). In many studies, it has been determined that CaO-NPs have high antibacterial and antibiofilm properties. Emamifar et al. (2010) prepared films containing silver and zinc oxide nanoparticles and used these films fort he storage of fresh orange juice. Packages prepared with solutions containing nanoparticles were filled with fresh orange juice and the samples were stored at 4°C. At the end of the study, it was determined that the storage life of the samples compared to fresh orange juice stored in conventional packages exceeded 28 days. As a result, it was reported that packages containing silver and zinc oxide nanoparticles could be an alternative packaging method.

CONCLUSION

Many methods are used to increase the shelf life of foods and to maintain their quality characteristics. Nanotechnological methods, one of these methods, are gaining interst day by day. Nanotechnology, which plays an important role in active packaging used to ensure that foods do not löse their quality characteristics during storage; especially the use of nanoparticles in packaging is effective on the shelf life of foods. Various nanoparticles (silver, zinc oxide, selenium, calcium oxide, gold, etc.) added to food packages Show both antioxidant and antimicrobial properties. The use of these nanoparticles, which are effective against various pathogens, in food packaging shows positive result in many aspects. Studies on this subject, which has an important potential in food packaging, should be increased and their effects in model food applications should be investigated.

REFERENCES

Almasi, H., Jahanbakhsh Oskouie, M., & Saleh, A. (2021). A review on techniques utilized for design of controlled release food active packaging. *Critical reviews in food science and nutrition*, *61*(15), 2601-2621.

Chandrasekar, C. M., Nespoli, L., Bellesia, T., Ghaani, M., Farris, S., & Romano, D. (2024). Fabrication of double layer nanoparticle infused starch-based thermoplastic food packaging system for meat preservation. International Journal of Biological Macromolecules, 254, 127689.

Cheng, H., Chen, L., McClements, D. J., Xu, H., Long, J., Zhao, J., ... & Jin, Z. (2024). Recent advances in the application of nanotechnology to create antioxidant active food packaging materials. *Critical Reviews in Food Science and Nutrition*, *64*(10), 2890-2905.

Cid-López, M. L., Soriano-Melgar, L. D. A. A., García-González, A., Cortez-Mazatan, G., Mendoza-Mendoza, E., Rivera-Cabrera, F., & Peralta-Rodríguez, R. D. (2021). The benefits of adding calcium oxide nanoparticles to biocompatible polymeric coatings during cucumber fruits postharvest storage. *Scientia Horticulturae*, *287*, 110285.

Dizaj, S. M., Lotfipour, F., Barzegar-Jalali, M., Zarrintan, M. H., & Adibkia, K. (2014). Antimicrobial activity of the metals and metal oxide nanoparticles. *Materials Science and Engineering: C*, *44*, 278-284.

Emamifar, A., Kadivar, M., Shahedi, M., & Soleimanian-Zad, S. (2010). Evaluation of nanocomposite packaging containing Ag and ZnO on shelf life of fresh orange juice. Innovative Food Science & Emerging Technologies, 11(4), 742-748.

Firouz, M. S., Mohi-Alden, K., & Omid, M. (2021). A critical review on intelligent and active packaging in the food industry: Research and development. *Food Research International*, *141*, 110113.

Gad, H. A., Alshubaily, F. A., Alsieni, M. A., Tayel, A. A., & Diab, A. M. (2022). Biosynthesis of nano-curcumin/nano-selenium composite and their potentialities as bactericides against fish-borne pathogens. *Green Processing and Synthesis*, *11*(1), 1098-1107.

Gandhi, N., Shruthi, Y., Sirisha, G., & Anusha, C. R. (2021). Facile and eco-friendly method for synthesis of calcium oxide (CaO) nanoparticles and its potential application in agriculture. *Saudi J. Life Sci*, *6*, 89-103.

He, X., Deng, H., & Hwang, H. M. (2019). The current application of nanotechnology in food and agriculture. *Journal of food and drug analysis*, *27*(1), 1-21.

Hosseini, S. F., Ghaderi, J., & Gómez-Guillén, M. C. (2022). Tailoring physico-mechanical and antimicrobial/antioxidant properties of biopolymeric films by cinnamaldehyde-loaded chitosan nanoparticles and their application in packaging of fresh rainbow trout fillets. *Food Hydrocolloids*, *124*, 107249.

Jamróz, E., Kopel, P., Juszczak, L., Kawecka, A., Bytesnikova, Z., Milosavljevic, V., & Makarewicz, M. (2019a). Development of furcellaran-gelatin films with Se-AgNPs as an active packaging system for extension of mini kiwi shelf life. *Food Packaging and Shelf Life*, *21*, 100339.

Jamróz, E., Kulawik, P., Kopel, P., Balková, R., Hynek, D., Bytesnikova, Z., ... & Adam, V. (2019). Intelligent and active composite films based on furcellaran: Structural characterization, antioxidant and antimicrobial activities. *Food Packaging and Shelf Life*, *22*, 100405.

Jeevanandam, J., Chan, Y. S., & Danquah, M. K. (2016). Biosynthesis of metal and metal oxide nanoparticles. *ChemBioEng Reviews*, *3*(2), 55-67.

Liu, J., Huang, J., Hu, Z., Li, G., Hu, L., Chen, X., & Hu, Y. (2021). Chitosan-based films with antioxidant of bamboo leaves and ZnO nanoparticles for application in active food packaging. *International Journal of Biological Macromolecules*, *189*, 363-369.

Singh, T., Shukla, S., Kumar, P., Wahla, V., Bajpai, V. K., & Rather, I. A. (2017). Application of nanotechnology in food science: perception and overview. *Frontiers in microbiology*, *8*, 1501.

Vera, P., Canellas, E., & Nerín, C. (2018). New antioxidant multilayer packaging with nanoselenium to enhance the shelf-life of market food products. *Nanomaterials*, *8*(10), 837.

Yildirim, S., Röcker, B., Pettersen, M. K., Nilsen-Nygaard, J., Ayhan, Z., Rutkaite, R., ... & Coma, V. (2018). Active packaging applications for food. *Comprehensive Reviews in food science and food safety*, *17*(1), 165-199.

CHEMICAL PROFILE AND ANTIOXIDANT ACTIVITIES OF THE HYDRO-METHANOLIC EXTRACT OF *PUNICA GRANATUM L.* PEELS

Taguigue Yasmine[1], *Becheker Imène*[1], *Laib Imen*[2]

1. Research Laboratory in Interactions, Biodiversity, Ecosystems and biotechnology. University 20 August, 1955, Skikda- Algeria. *E-mail : y.taguigue@univ-skikda.dz ; imene.becheker@gmail.com*
2. BIOQUAL Laboratory, Institute of Nutrition, food and Agro-food Technologies (I.N.A.T.A.A). Mentouri Brothers University, Constantine-Algeria. *mina.laib@gmail.com*

ABSTRACT

In this paper, the LC-ESI-MS/MS method is used to establish the phytochemical profile of the hydro-methanolic extract of pomegranate peels. Antioxidant activity was determined by two methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) against BHA and BHT standards and Ferric reducing antioxidant power assay FRAP against ascorbic acid and alpha tocopherol standards**.** The findings indicate the abundance of the hydro-methanolic peel extract in phenolic compounds where we could identify 22 compounds with a higher content of Riboflavin, Epicatechin, Oleanolic acid, Naringenin and p-Coumaric acid. The extract presents a substantial antioxidant activity at low concentrations in which we recorded an IC_{50} value of 5.79±0.15 µg/ml for DPPH and an $A_{0,5}$ value of 1.49±0.05 µg/ml for FRAP.
This study showed that the peel extract of *Punica granatum L* is an excellent antioxidant due to its richness in phenolic compounds. As a result, it opens up prospects in pharmaceutical and nutraceutical applications.

Key words: DPPH, FRAP, Hydro-methanolic extract, LC-ESI-MS/MS, *Punica granatum L*.

INTRODUCTION

Pomegranate is an ancient fruit originated from Iran and the Mediterranean basin belonging to the Lytheracea family REF. It is a shrub that produces a small ball shape fruits with red sweet arils from the inside and crunchy white seeds. Not only consumed as a fruit and molasses but also utilized historically as a remedy by using its bark in folk medicine to threat inflammations and gastric ulcers REF. The aim of this paper is to shed light on the beneficial effect of the pomegranate peel extract, its composition in bioactive compounds and its antioxidant capacity.

MATERIAL AND METHODS

- **Collection and preparation of the fruit (biological) material**

Fresh pomegranate fruits were collected from the local market, washed with tap water and peeled then the peels were cut into small pieces and let dried completely in shade after that they were grinded into powder and stocked in glass jars away from light till utilization.

- **Preparation of hydromethanolic extract**

100mg of the peel powder was weighted and a solution of methanol/water (70:30) was added. The mixture was kept in the dark for 48h with solvent renewal every 24h and under stirring. The solution was filtered through a filter paper. The filtrate was evaporated in a rotary evaporator at 50°C to obtain the crude extract.

- **Sample preparation for the LCMS analysis**

In order to optimize the samples for the LCMS/MS analysis and minimize the interferences a solid phase extraction (SPE) is a necessary step. First, about 1mg of the crude extracts were diluted in 1ml of methanol/water mixture, vortexed and centrifuged. After that, the samples were loaded in a chromatography column (ISOLUTE C18) then the lipids were eliminated with a hexane wash. Finally, the targeted molecules were eluted in clean tubes with methanol and filtered through a nylon syringe microfilters membrane of 13mm diameter, a pore size of 0,22µm. 100µl of the filtered solution is diluted in 1ml methanol.

- **Liquid chromatography-electrospray ionization-mass spectrometry analysis**

The LC-ESI-MS analysis is carried out in an UPLC system coupled with a tandem mass spectrometry and an ESI electro-spray ionization Shimadzu 8040 Ultra-High sensitivity with UFMS (Ultra-Fast Mass Spectrometry) technology and equipped with binary bump Nexera XR LC-20AD.

The mobile phase is used in the gradient mode on a Restek Ultra C_{18} column with 3µm diameter and 150x4.6mm dimensions and a fixed temperature of 40°C. The solvent A is water and 0,1 % formic acid and the solvent B is methanol. The flow rate is 0,2ml/min and the injection volume is 5 µL. The gradient elution and the conditions for electro spray ionization (ESI) were as follows:

0 min to 0.2min A 98 %
0.2 min to 7.5min A 25%
7.5 min to 12.5min A 0%
12.5 min to 17min A 0%
17 min to 18min A 98%
18 min to 21min A 98%

CID gas, 230 KPs; conversion dynode, −6.00 Kv◦C; DL temperature, 250 ◦C; nebulizing gas flow, 3.00 L/min; heat block, 400 ◦C; drying gas flow, 10L/min.

- **In vitro antioxidant activity measurement**

1. **DPPH free radical scavenging assay**

The free radical scavenging activity was determined according to **(Blois 1958)**. A stock solution of DPPH at 4% (4mg of DPPH in 100ml of methanol) stocked at -20°C in the dark and adjusted at 0,7 at 517nm. 40µl of the extract was mixed with 160µl of the stock solution. Results were read at 517nm in a microplate reader after 30min of incubation. Findings were compared with the antioxidant standards Butyl Hydroxy Anisole (BHA) and Butyl Hydroxy Toluene (BHT) and were expressed in 50% of the inhibition concentration in µg/ml (IC_{50}).

2. **Ferric reducing antioxidant power assay FRAP**

The ferric reducing antioxidant power activity was determined by the method of Oyaizu (1986) with slight modification. A solution of tri-chloro-acetic acid (TCA) at 10% was prepared by dissolving 1g of TCA in 10ml of distilled water. The reaction is carried out by mixing 10µl of the extract with 40µl of the phosphate buffer (pH 6,6) and 50µl of potassium ferricyanide Prussian red ($K_3Fe\ (CN))_6$ at 1%. The mixture was incubated at 50°C for 20mn then 50µl of TCA solution, 40µl of distilled water and 10µl of ferric chloride ($FeCl_3$) at 0,1% were added. The reading is done at 700nm. Results were compared with the antioxidant standards alpha tocopherol and ascorbic acid and were expressed as $A_{0,5}$ which indicates 50% of the inhibition concentration.

- **Statistical analysis**

All the experimental results are mentioned as a mean ± standard deviation of three trials.

Results and discussion

- **Liquid chromatography-electrospray ionization-mass spectrometry analysis**

According to the literature the pomegranate peel extract is reach in bioactive compounds with a wide range of polyphenols and flavonoids REF. In this study we were able to identify 22 different compounds by the LCMS analysis represented in **(Table1)** in which phenolic acids, flavonoids, phenolic aldehyde and vitamins and with a higher amount of Riboflavin, Epicatechin, Oleanolic acid, Naringenin and p-Coumaric acid respectively.

Table1: Phytochemical profile of *Punica granatum L*

ID #	Name	Molecular Formula	Molecular Weight	ESI Charge (+/-)	m/z	Ret. Time	Height	Area
1	Folic Acid	C19H19N7O6	441	(+)	442.9000>59.1000	12.496	546	3260
2	beta carotene	C40H56	536.87	(+)	537.2000>23.1000	15.510	7001	39613
3	Curcumin	C21H20O6	368.4	(+)	368.9000>145.0500	7.329	4949	31392
4	Cinnamic acid	C9H8O2	148.16	(+)	149.0500>131.0000	10.176	837	7212
5	Epicatechin	C15H14O6	290.27	(+)	290.9000>139.0500	7.193	67239	351035
6	Thymol	C10H14O	150.22	(+)	151.1000>109.0500	11.530	1931	9036
7	Luteolin	C15H10O6	286.24	(+)	286.7500>153.0000	9.751	7245	33098
8	Chrysin	C15H10O4	254.24	(+)	255.1000>68.8500	13.031	1463	6778
9	Oleanolic Acid	C30H48O3	456.7	(+)	457.3000>411.5000	17.081	25850	149385
10	acide maleic	C4H6O5	134.09		117.1000>85.2000	3.803	2225	6256
11	Oleauropin	C25H32O13	540.5	(-)	541.3000>146.4000	5.074	7150	20890
12	Riboflavin	C17H20N4O6	376.4	(+)	377.9000>361.3500	14.711	4764762	34776451
13	Rutin	C27H30O16	610.5	(+)	611.0000>465.2000	9.199	9823	57484
14	Sinapic Acid	C11H12O5	224.21	(+)	225.0000>91.1000	10.149	4000	18742
15	Syrinjic Acid	C9H10O5	198.17	(+)	199.0000>140.1000	7.580	3388	13462
16	ferulic acid	C10H10O4	194.18	(+)	194.8000>177.0500	7.812	4991	29166
17	Naringenin	C15H12O5	272.25	(+)	273.0500>153.0000	9.331	17644	82350
18	Kampferol	C15H10O6	286.24	(+)	285.0000>93.0000	11.177	445	1386
19	3.5-dihydroxybenzoic Acid	C7H6O4	154.12	(-)	153.0000>109.0500	1.559	3859	12309
20	Caffeic Acid	C9H8O4	180.16	(-)	179.1500>135.0000	1.686	3668	11532
21	gallic acid	C4H4O4	170.12	(-)	169.0000>124.9000	3.481	8430	67476
22	p-Coumaric Acid	C9H8O3	164.16	(-)	163.0500>118.9500	8.678	14511	82055

- **In vitro antioxidant activity measurement**

The value of IC_{50} registered for the DPPH assay was 4,65±0,48 µg/ml compared to BHA and BHT standards (6,14±0,41and 12,99±0.41 µg/ml) and the value of $A_{0,5}$ registered for the FRAP assay was 1,49±0,05µg/ml compared to ascorbic acid and α–tocopherol standards (6.77±1.15 and 34.93±2.38 µg/ml) (Table2) which represent an excellent antioxidant capacity of the *Punica granatum.L* peel extract.

Table2: Antioxidant activity thehydromethanolic extract of *Punica granatum L* peels

	Punica granatum .L extracts	BHA	BHT	Alpha tocopherol	Ascorbic acid
DPPH assay IC_{50} (µg/ml)	4,65±0,48	6,14±0,41	12,99±0.41	NT	NT
Reducing power assay $A_{0.5}$ (µg/ml)	1,49±0,05	NT	NT	34.93±2.38	6.77±1.15

BHA: butyl hydroxy anisole, BHT: butyl hydroxy toluene, NT: not tested

CONCLUSION

The hydromethanolic extract of *Punica granatum.L* showed a very high antioxidant activity by the two methods tested and phytochemical screening confirms the abundance of multiple bioactive compounds. As a result, it opens up prospects in pharmaceutical and nutraceutical applications.

REFERENCES

1. Shaygannia E, Bahmani M, Zamanzad B, Rafieian-Kopaei M. A Review Study on Punica granatum L. Journal of Evidence-Based Complementary & Alternative Medicine 2016;21(3):221-227. doi:10.1177/2156587215598039
2. Jurenka, Julie S.Therapeutic Applications of Pomegranate (Punica granatum L.): A Review. Alternative Medicine Review, 2008, Vol 13, Issue 2, p128
3. M. Oyaizu, Japanese Journal of Nutrition. 307(1986)315.
4. M.S. Blois, Nature. 1119(1958)1200.
5. S. Zerrouki, S. Mezhoud, C. Bensouici, O. Boumaza, R. Seghiri and R. Mekkiou. Phenolic and flavonoid contents and antioxidant activity of Tourneuxia variifolia Coss. extracts (Asteraceae) Journal of New Technology and Materials (JNTM) J. New Technol. Mater. Vol. 12, N°02 (2022)97-100

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OIL EXTRACTED FROM WASTE OF *JUNIPERUS COMMUNIS* L. MEDICINAL AND AROMATIC PLANTS INDUSTRY IN ALBANIA

ELTON BASHA[1,*], *ERJON MAMOÇI* [2], *ENTELA HODAJ-ÇELIKU*[3], *ANIKET SHARMA*[4], *BLEDAR BISHA*[4]

[1] Department of Agri-Food Technology, Agricultural University of Tirana, Tirana, Albania.
[2] Department of Food Science and Biotechnology, Agricultural University of Tirana, Tirana, Albania.
[3] Department of Chemistry, Agricultural University of Tirana, Tirana, Albania.
[4] Department of Animal Science, University of Wyoming, Wyoming, United States of America.

* Correspondence: ebasha@ubt.edu.al

The Medicinal and Aromatic Plants (MAPs) industry is relevant for Albania covering approximately 20% of agricultural exports. Mostly high quality dried parts of MAPs are traded, while products not fulfilling quality criteria and non-tradable plant parts are often regarded as waste products, even though they contain valuable bioactive substances. This study evaluates the composition and biological activity of the essential oil (EO) fraction of wastes generated from the MAPs industy of *Juniperus communis* L. in Albania.
Juniperus communis L. was collected from Korçë area, dried, screened for trade quality berries in an industrial plant for MAPs, and the waste parts underwent hidrodistilation in industrial distilators. Chemical composition was performed using Gas Chromatography coupled with Mass Spectrometry and identified 50 compounds, where main components were α-Pinene (24.47%), Sabinene (12.4%), Germacrene D (3.2%) and β-Myrcene (1.6%). The antioxidant capacity of *J. communis* L.EO was determined by 2,2-diphenyl-1-picrylhydrazil (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and expressed as Inhibitory Concentration of 50% of the free radical (IC_{50}) where values were IC_{50}= 155.4 µg/mL and IC_{50}= 163.2µg/mL for DPPH and ABTS respectively. Antimicrobial activity of *J. communis* L. EO was determined against five bacteria, *Escherichia coli ATCC 10535; Salmonella enteritidis, ATCC 49223, Pseudomonas aeruginosa, ATCC 9027; Micrococcus luteus, ATCC 10240; Stenotrophomonas maltophilia, ATCC 1363;*and one yeast, *Candida albicans, ATCC 10231* by microdilution method used to determine the minimum inhibitory concentration (MIC). The EO showed no antimicrobial activity against the first 3 bacterial strains, while it inhibited growth of *Micrococcus luteus* and *Candida albicans* at concentrations of 2.5 mg/mL and of *Stenotrophomonas maltophilia* at the maximum tested concentration of 5 mg/mL.

Keywords: *Juniperus communis* L , *antioxidant, antimicrobial, MIC*

1. INTRODUCTION

Albania has a long tradition in the production and export of medicinal and aromatic plants (MAPs), spanning more than 60 years, which is mainly based on the abundance of wild-grown MAPs. Compared to other agricultural subsectors, MAPs industry is primarily focused on exports, with over 95% of all MAPs exported, accounting for 20% of all agricultural exports (AASF, 2019). A considerable amount of post-harvest waste products are produced during the processing of MAPs, such as branches, leaves and fruits of lower quality (Routray. *et al.*, 2017), that are not suitable for commercial use (FAO/WHO Codex Alimentarius Commission On Spices and Culinary Herbs, 2021). Since these residual biomasses have the same components and characteristics as the finished product, they could be sources of bioactive chemicals (Navarrete *et al.*, 2011; Wang *et al.*, 2018). Most of these by-products are still treated as waste from the Albanian MAPs industry and discarted improperly or used as a burning material. Nevertheless MAPs by-products are a source of useful metabolites with significant biological characteristics that might give to finished products a unique quality (Sahaa. *et al.*, 2020).
One of the most prized aromatic plants in the world is juniper (*Juniperus* spp.), found also in Albania in two main species, red juniper (*Juniperus oxycedrus* L.) and black juniper (*Juniperus communis* L.) which possess antioxidant, anti-inflammatory, and antimicrobial properties due to the occurrence of several secondary metabolites, such as phenolic constituents, terpenoids, and flavonoids, in their extracts (Mërtiri *et al.*, 2024). Both spieces are employed for culinary and also essential oil (EO) uses, but there is a higher demand for the second, black juniper, in the Albanian MAPs industry. Also *J. communis* has been sold at higher prices in recent years in Albania due to domestic and international supply dynamics and higher domestic demand for essential oil production (Medicinal and Aromatic Plants Sector Study, 2021).
However, considerable quantities of *J. communis* herbal residues are formed during the post-harvest processing used to select the material to be marketed, and these residues can be used to extract its EO. While there are various ways to extract natural essential oils (EOs) from plants, steam distillation is the method most commonly employed in the industry today since it is easy to use and doesn't require the use of chemicals like solvents, which are often utilized in other extraction processes (Esteban *et al.*, 2023).

Due to the current issue with pathogenic bacteria whose resistance to antibiotics has grown over time, one of the main problems facing the pharmaceutical, food, and veterinary industries is discovering bioactive compounds to be employed as bactericides (Mancuso *et al.*, 2021). As many active organic compounds make up plant EOs, which act on microbial cells through different mechanisms, they do not induce microbial resistance (Rao *et al.*, 2019). Additionally, since customers are increasingly requesting natural products as alternatives to synthetic preservatives, it is necessary to look for antioxidant and anti-inflammatory active principles of natural origin. In this context, the US Code of Federal Regulation has classified juniper berries, essential oils, solvent-free oleoresins and natural extractives as Generally Recognized as Safe (GRAS) for their intended application (Code of Federal Regulation. Substances Generally Recognized as Safe, 2023).

The aim of this study was to characterize the chemical composition and assess the biological activity of the EO extracted from the by-products of *J. communis* MAPs industry, as a strong source of bioactive components for the creation of new products in food and pharmaceutical industry.

2. MATERIALS AND METHODS

2.1 Plant material and EO extraction

Juniperus communis L. was collected from Korçë district, Albania, from 10th of August until the end of September 2021, Albania, dried, screened for trade quality berries which are mainly intended for gin spirit and teas production in an industrial plant for MAPs (PETKUS Technologie GmbH), and the remaining by-product underwent hidrodistilation in industrial distilators at BioBes sh.p.k. company (Sopëz, Albania).

2.2 Reagents and microorganisms

Antioxidant radical screening reagents [20-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium and potassium persulfate (ABTS)] were purchased from Alfa Aesar (Massachusetts, United States). Methanol was secured from VWR International (Fontenay-sous-Bois, France) and ethanol fromMerk KGaA (Darmstadt, Germany).

The bacterial and fungal ATCC strains [American type culture collection strain (ATCC) *Salmonella enteritidis* (ATCC:49223), *Escherichia coli* (ATCC:10535), *Pseudomonas aeruginosa* (ATCC:9027), *Stenotrophomonas maltophilia* (ATCC:13637). *Micrococcus luteus* (ATCC:10240), along with one fungal isolate *Candida albicans* (ATCC:10231) were procured from Microbiologics, Inc., (MinnesotaUnited States). 96-well plates were secured from Corning Inc. (New York, United States).

Blood agar medium and Muller Hinton Broth were procured from Remel Inc, (California, United States), 0.5 Polymer McFarland Standard from Thermo Fisher Scientific (Massachusetts, United States) and Dimethylsulfoxide (DMSO) from Sigma-Aldrich (Missouri, United States).

2.3 Gas Chromatography-Mass Spectrometry

Gas Chromatography-Mass Spectrometry Essential oil analyses were performed on a Shimadzu GC-2010-GCMSQP2010 system operating at 70 eV. The temperature program was from 60ºC to 250ºC, at a rate of 5ºC/min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Injection volume of each sample was 1 μL. Retention indices for all compounds were determined according to Van den Dool and Kratz, 1963, using n-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of NIST21 and NIST107, Massada, 1976 and by comparison of their retention indices with literature data Adams, 2007. Component relative concentrations were calculated based on GC peak areas without using correction factors. Essential oils were often subjected to co-chromatography with authentic compounds (Fluka, Sigma).

2.4 Antioxidant activity

The free radical scavenging activity of the essential oils was measured *in vitro* by the 2,20-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium and potassium persulfate (ABTS) assay according to Brand-Williams *et al.*, (1995) and to Re *et al.*, (1998) respectively. The stock solution for the DPPH assay was prepared by dissolving 24 mg of DPPH with 100 mL of methanol (and stored at 20°C. The working solution was obtained by diluting the DPPH stock solution with methanol to achieve an absorbance of about 0.98±0.02 at 517 nm using a spectrophotometer (Biochrom Ltd. Libra S22). The stock solution for the ABTS assay was prepared by dissolving ABTS in water at a concentration of 7 mM. The ABTS radical cation (ABTS•1) was obtained by combining ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and thus allowing the mixture to stand in the dark at room temperature for 12–16 h before use. The working solution was obtained by diluting the ABTS stock solution with ethanol to achieve an absorbance of about 0.7±0.02 at 734 nm using a spectrophotometer.
Three ml portions of these solutions were mixed with 100 µL of the sample at 6 concentrations: 0.5, 1, 2, 5, 10, 20 mg/mL. The mixture was shaken well. For the DPPH assay it was incubated in the dark for 30 min at room temperature assay and then the absorbance was measured at 517 nm. For the ABTS assay the absorbance was measured at 734 nm after 5-6 minutes. The controls were prepared as above but without essential oil. The activity was evaluated based on the percentage of the DPPH and ABTS radical removed as the following equation:

Scavenging activity in % = {(absorbance of control - absorbance of sample) / (absorbance of control)} x 100.

Result was calculated as the concentration of essential oil that inhibits 50% of the free radical (Inhibition Concentration IC_{50}).

2.5 Evaluation of antimicrobial activity by Micro-dilution Broth Experiment

The Essential Oil (EO) antimicrobial activities were investigated against different clinical and food-borne pathogens using standard American type culture collection strain, five bacteria along with one fungal isolate. . The bacterial and fungal ATCC strains were stored at 4°C and sub-cultured for the experimental setup. Prior to the inoculation of the

strains with EOs, the microorganisms were grown at 28 or 37 °C (for fungus and for bacteria) for 18-20 h on blood agar medium.
The MIC (Minimum Inhibitory Concentration) of each EO was determined using a broth microdilution method in 96-well plate in accordance with the Clinical & Laboratory Standards Institute (CLSI) protocols [M100 Ed34, 2024]. In brief, bacterial suspensions were adjusted to a final concentration of 10^5 CFU/mL cells standardized by 0.5 McFarland in Muller Hinton Broth (MHB) media. EO stock concentration of 100 mg/ml was prepared by dissolving in DMSO. From this stock, a working concentration of 5 mg/ml was prepared (in MHB media) to be used in plate susceptibility testing, ensuring DMSO concentration less than 5%. This resulted in EO's concentration range of 5mg/ml to 0.0097 mg/ml on the plate. One hundred microliters of bacterial suspension were finally added to each well. The plate setup included the 11^{th} column as the media control (negative control), and the 12th column containing bacteria and media (positive control). Additionally, rows D and E were designated for solvent controls, with row D with only EO (solvent control) and row E for DMSO (conc. used to dissolve EO) and bacteria served as DMSO control. The plates were incubated at 37°C for 24 h. MIC was determined using Tecan I-control software (Infinite M Plex TECAN) to measure the OD (600nm) compared with positive control. Everything was kept constant for determining antifungal activity except incubation was done for 45-48h, with OD determined at 530nm. MIC was determined as the lowest concentration of EO that inhibited visible growth of the tested microorganism (Puškárová A *et al.* 2017).

3. RESULTS AND DISCUSSION

3.1 Plant material and EO extraction

The amount of by-product from different batches ranged from 10 to 20% of the total mass of *J. communis* L, with a mean value of 13%.The by-product is mainly composed of niddles and few berries on the top of the branch. The industrial hydrodistillation employed to extract the EO from *J. communis* L. by-product yielded 0.8% of the total mass of the plant material and the EO resulted in white-yellow colour.

3.2 Quantitative and qualitative analysis of the EO

GC-MS analysis (Figure 1) identified in total 49 different components, counting for 86.4% of the EO. Results are given in Table 1, where compound are listed according to their Arithmetic Index. The oil was composed mainly by the monoterpene fraction (62.3%) with α-Pinene (24.47%) being the most abundant compound, followed by Sabinene (12.4%), γ -Terpinene (4.6%), Limonene (4.1%). The sesquiterpene compounds constituted 24.1% of the essential oil with β-Caryophyllene (3.9%) and Germacrene B (3.8%) as main compounds. In general the EO composition was in compliance with ISO 8879 and The European Pharmacopoeia. 10th edition (Eur.Ph. 10^{th}) specifications, exept for α-Pinene being slightly lower than the specification of ISO 8879, and α-Phellandrene being above the maximum level set by Eur.Ph. 10th.

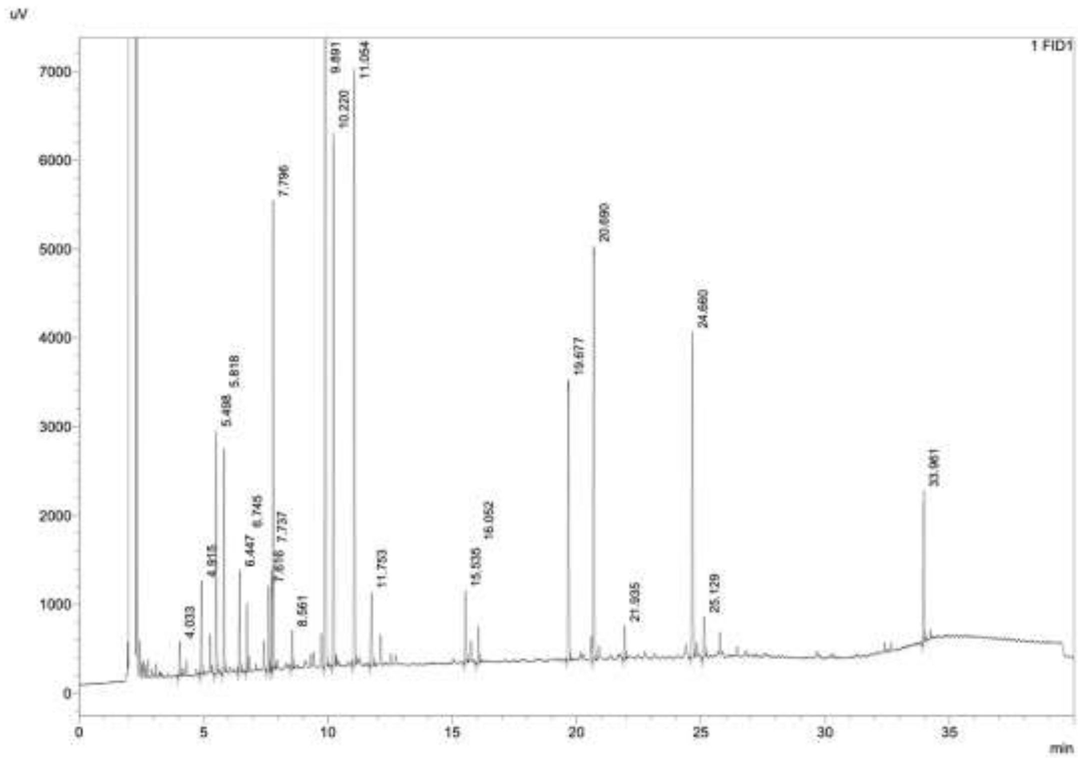


Figure 1. GC-MS chromatogram of EO from *J. communis*L. by-product.

Main components were in agreement with other reports from Albania (Salamon *et al.*, 2014; Buci *et al.*, 2018, Agastra *et al.*, 2021) and from other countries (Angioni *et al.*, 2003; Höferl *et al.*, 2014; Koukos *et al.*,1999) except for the concentration of β-Myrcene, where it is generally reported in higher concentrations as one of the main components. Similar β-Myrcene levels were found by Ainan *et al.*, (2022) with β-Myrcene *J. communis* L. harvested in Maroco. Probably this is due to high levels of leaves (niddles) in the by-product from *J. communis* L as the primary component that distinguishes berry oil from needle oil is myrcene, which may be found in berry oil at levels as high as 20% whereas it can only be found in needle oil at levels as low as 5% (Shamir *et al.*, 2003).

Table 1. Composition of the essential oil from *J.communis*L. by-product and reference values obtained for specific compounds considered in ISO 8879 and Eur.Ph. 10th edition.

Compounds [a]	**AI** [b]	**Percentage** (%)	**ISO 8897**	**Eur. Ph. 10th**	**ID** [c]
Tricyclene	919	1.1			AI, MS
α-Thujene	926	3.3			AI, MS
α-Pinene	931	24.47	25-45	20-50	AI, MS, Co-GC
Camphene	945	0.3			AI, MS
Thuja-2,4(10)-diene	952	0.2			AI, MS
Sabinene	972	12.4	4-20	max. 20	AI, MS
β-Myrcene	992	1.6	3-22	1-35	AI, MS, Co-GC
α-Phellandrene	1003	1.5		max. 1	AI, MS
p-Cymene	1024	2.7			AI, MS, Co-GC
Limonene	1027	4.1	2-8	2-12	AI, MS
γ-Terpinene	1059	4.6			AI, MS, Co-GC
Terpinolene	1087	3.1			AI, MS
Monoterpene hydrocarbons 59.37 %					
Linalool	1101	0.08			AI, MS, Co-GC
α-Campholenal	1126	0.08			AI, MS
trans-Pinocarveol	1138	0.07			AI, MS
Borneol	1164	0.2			AI, MS, Co-GC
Terpinene-4-ol	1176	1.6	1-6	0.5-10	AI, MS, Co-GC
p-Cymen-8-ol	1187	0.1			AI, MS
α-Terpineol	1191	0.06			AI, MS
Citronellol	1232	0.15			AI, MS
Bornyl acetate	1286	0.15	n.d-0.6	max. 2	AI, MS, Co-GC
Undecanone	1297	0.14			AI, MS
α-Cubebene	1349	0.1			AI, MS
Citronellyl acetate	1357	0.2			AI, MS
Oxygenated Monoterpenes		**2.93 %**			
α-Ylangene	1371	0.1			AI, MS

α-Copaene	1375	0.1			AI, MS
β-Caryophyllene	1419	3.9		max. 7	AI, MS, Co-GC
β-Copaene	1428	0.3			AI, MS
γ-Elemene	1434	0.6			AI, MS
Aromadendrene	1438	0.5			AI, MS
Cis-Muurola-3,5-diene	1450	0.1			AI, MS
α-Carryophyllene	1453	1.4			AI, MS, Co-GC
trans-Cadina-1 (6), 4-diene	1473	0.2			AI, MS
γ-Muurolene	1477	1.4			AI, MS
Germacrene D	1481	3.2	1-5		AI, MS
β-Selinene	1486	0.4			AI, MS
trans-Muurola-4(14),5-diene	1492	0.1			AI, MS
Viridiflorene	1495	0.9			AI, MS
α-Muurolene	1500	0.7			AI, MS
δ-Amorphene	1510	0.1			AI, MS
γ-Cadinene	1514	1.6			AI, MS
δ-Cadinene	1524	1.2	1-3.5		AI, MS
Germacrene B	1557	3.8			AI, MS
Sesquiterpene Hydrocarbons		**20.6 %**			
Spathulenol	1578	0.3			AI, MS
Humulene epoxide II	1610	0.4			AI, MS
1-epi-Cubenol	1629	0.6			AI, MS
α-Muurolol (Torreyol)	1643	0.8			AI, MS
Cubenol	1648	0.6			AI, MS
α-Cadinol	1656	0.8			AI, MS
Oxygenated Sesquiterpenes		**3.5 %**			
Total		**86.4 %**			

[a]Compounds listed in order of elution from an HP-5 MS capillary column; [b] AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C23); [c] Identification method: AI=Arithmetic Index, MS=mass spectrum, Co-GC=Coinjection with authentic compound. Concentrations below 0.05% are marked as tr (traces).

3.3 Antioxidant activity

DPPH assay was one of the *in vitro* tests used in this study to determine the ability of juniper berry oil components to act as hydrogen atom donors. The EO Juniper by-product oil was a weak DPPH radical reducer with IC_{50} value of 155.4 µg/mL. This was expected as the main component in the EO, α-pinene, was found by Emami *et al.*, (2007) to show no antioxidant activity at all in relation to DPPH radicals. The same work established that γ-terpinene (17.74%) showed antiradical activity in relation to DPPH radicals. Also limonene in 10–50 µg/mL concentrations causes DPPH inhibition from 16% to 25% (Roberto *et al.*, 2010).
In relation with the ABTS radicals the result was similar with an IC_{50} value of 163.2µg/mL. The results are shown in table 2.

Table 2. Antioxidant activity of essential oil from *J. communis* L. by-product by DPPH and ABTS assays.

Assay	**IC_{50}*** *J. communis* **L. EO**	***St. Dev***
DPPH	155.4 µg/mL	±7,04
ABTS	163.2 µg/mL	±8,78

3.4 Antimicrobial activity

Table 3 shows the antibacterial activity of essential oil from *J. communis* L. by-product as Minimum Inhibitory Concentration. In tested concentrations the oil was not active against *E. coli, S. enteritidis* and *P. aeruginosa* bacterial strains while it resulted active against *M. luteus* and *S. maltophilia* with MIC 2.5 and 5mg/ml respectively. Similar results were reported from Sela *et al.*, (2013) where *J. communis* leaves and berries EO using broth dilution method showed no activity against *E. coli, S. enteritidis* and *P. aeruginosa*. The oil showed antifungal activity against *C. albicans* yeast with MIC 2.5 mg/ml. However According to Sela *et al.*, (2013) no MIC could be measured against *C. albicans* yeast from *J. communis* leaves and berries EO. Similar results were obtained from Angioni *et al.*, (2003) were *J. communis* EO tested was not active against *C. albicans*. To our knowledge this is the first report that shows the antibacterial activity of essential oil from *J. communis* on *S. maltophilia.*

Table 3. Minimum Inhibitory Concentration of essential oil from *J. communis* L. by-product.

Microorganism	**MIC *Juniperus communis* L. antimicrobial activity**
E. coli ATCC 10535	NO MIC
S. enteritidis ATCC 49223	NO MIC
P. aeruginosa ATCC 9027	NO MIC
M. luteus ATCC 10240	2.5 mg/ml
S. maltophilia ATCC 13637	5 mg/ml
C. albicans ATCC 10231	2.5 mg/ml

4. CONCLUSIONS

The purpose of this study was to assess the essential oil that was industrially extracted from *J. communis*L. by-product that was produced by the MAP industry by analyzing its chemical content and biological activity, in order to determine the potential value of this by-product.

The chemicals' presence is consistent with findings from the literature. Furthermore, proof of these compounds' existence in the EO supports the moderate free scavenging activity ascertained by the DPPH and ABTS assay, which is similar to EO isolated from *J. communis* L. primary material. Therefore, this industry by-product is appropriate to be used to produce EO that meets the general requirements for *J. communis* L. EO as established by ISO 8879 and Eur. Ph. 10th.

The terpenes and other volatile constituents found in the by-product plant material could be further utilized for the production of enriched aromatic beverages in which *J. communis* L finds wide applications (Enescu *et al.* 2016).

Considering all these findings, it is clear that the by-product plant material is a valuable source of bioactive compounds and that it could be used to produce novel nutritional products by as antioxidant, antimicrobial and flavoring additives to food.

5. REFERENCES

AAFS (2019). Medicinal and Aromatic Plants Sector Study. Albania Agribusiness Support Facility (AASF). Institute of Economics Studies and Knowledge Transfer Tirana, 2019. Available online at: https://aasf.com.al/wp-content/uploads/2019/08/Map-EN.pdf

Sela, F.; Karapandzova, M.; Stefkov, G.; Cvetkovikj, I.; Trajkovska-Dokik, E.; Kaftandzieva, A.; Kulevanova, S. Chemical composition and antimicrobial activity of leaves essential oil of Juniperus communis (Cupressaceae) grown in Republic of Macedonia. Maced. Pharm. Bull. 2013, 59, 23–32.

Adams R.P.,(2007) Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. In: Forth Edition. Allured Publishing Corporation, Carol Stream, IL.

Agastra, A.; Gixhari, B.; Kadiasi, N.; Ibraliu, A. Influence of environmental factors in the composition of essential oils content ofJuniperus communis L. berries, in Southeast part of Albania. Int. J. Ecosyst. Ecol. 2021, 11, 943–948.

Ainane, A., Abdoul-Latif, F. M., Ali, A. M., Mohamed, J., Shybat, Z. L., & Ainane, T. (2022). Chemical composition ofJuniperus communis L. essential oil and evaluation of its antifungal activity in vitro against Ascochyta rabiei. Journal ofAnalytical Sciences and Applied Biotechnology, 4(2), 108-115.

Angioni A, Barra A, Russo MT, Coroneo V, DessiS, Cabras P. Chemical composition of the essentialoils of Juniperus from ripe and unripe berriesand leaves and their antimicrobial activity. J AgricFood Chem 2003; 51(10):3073-3078. doi:

Brand-Williams W, Cuvelier ME, Berset C: Use of free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und-Technol 1995, 28:25–30.

Buci, A.; Hodaj Celiku, E.; Manaj, H.; Abazi, S.; Drushku, S.; Lazari, D. Essential oilcomposition from Juniperus communis originated from Albania. Int. J. Environ. Appl. Sci.2018, 13, 15-19.

Code of Federal Regulation. 21 CFR Part 182–Substances Generally Recognized as Safe (4–1–23 Edition). 2023. Availableonline: https://www.govinfo.gov/content/pkg/CFR-2023-title21-vol3/pdf/CFR-2023-title21-vol3-part182.pdf

Damjanowić B, Skala D, Baras J, Petrović-DjakovD. Isolation of essential oil and supercritical carbondioxide extract of Juniperus communis L fruitsfrom Montenegro. Flavour Fragr J 2006; 21:875-880. doi:

Emami, S.A.; Javadi, B.; Hassanzadeh, M.K. Antioxidant activity of the essential oils of differentparts of Juniperus communis. subsp. hemisphaerica and Juniperus oblonga. Pharm. Biol. 2007,45, 769–776.

Enescu, C.M.; Houston Durrant, T.; Caudullo, G.; de Rigo, D. Juniperus communis in Europe: Distribution, habitat, usage and threats. In European Atlas of Forest Tree Species; San-Miguel-Ayanz, J., de Rigo, D., Caudullo, G., Houston Durrant, T., Mauri, A., Eds.; Publications Office of the EU: Luxembourg, 2016; p. e01d2de+.

Esteban LS, Mediavilla I, Xavier V, Amaral JS, Pires TCSP, Calhelha RC, López C, Barros L. Yield, Chemical Composition and Bioactivity of Essential Oils from Common Juniper (Juniperus communis L.) from Different Spanish Origins. Molecules. 2023 May 30;28(11):4448.

Höferl M, Stoilova I, Schmidt E, Wanner J, Jirovetz L, Trifonova D *et al.* Chemical compositionand antioxidant properties of Juniper berry(Juniperus communis L.)

essential oil on the antioxidantprotection of Saccharomyces cerevisiaemodel organism. Antioxidants 2014; 3:81-98.

Joint FAO/WHO Codex Alimentarius Commission. Codex Alimentarius:CX/SCH 21/5/3 On Spices and Culinary Herbs. Rome: World HealthOrganization: Food and Agriculture Organization of the United Nations (2021).

Koukos PK, Papadopoulou KI. Essential oil of Juniperuscommunis L. grown in Northern Greece:variation of fruit yield and composition. J EssentOil Res 1997; 9:35-39. doi:

M100 Ed34 | Performance Standards for Antimicrobial Susceptibility Testing, 34th Edition. Clinical & Laboratory Standards Institute. https://clsi.org/standards/products/microbiology/documents/m100/

Mancuso, G.;Midiri, A.; Gerace, E.; Biondo, C. Bacterial Antibiotic Resistance: TheMost Critical Pathogens. Pathogens 2021, 10, 1310.

MassadaY., (1976) Analysis of essential oils by Gas Chromatography and Mass Spectrometry: John Wiley & Sons, New York.

Medicinal and Aromatic Plants Sector Study. Selected sectorial analysis as a solid ground for the preparation of IPARD III programme and of Strategy for Agriculture, Rural Development andFishery 2021-2027 for Albania. May 2021.

Mërtiri, I.; Păcularu-Burada, B.; Stănciuc, N. Phytochemical Characterization and Antibacterial Activity of Albanian Juniperus communis and Juniperus oxycedrus Berries and Needle Leaves Extracts. Antioxidants 2024, 13, 345.

Navarrete A, Herrero M, Martin A, Cocero MJ. Ibanez, E. Valorizationof solid wastes from essential oil industry. J Food Eng. (2011) 104:196–201.

Puškárová A, Bučková M, Kraková L, Pangallo D, Kozics K. The antibacterial and antifungal activity of six essential oils and their cyto/genotoxicity to human HEL 12469 cells. Sci Rep 2017;7:8211.

Rao J, Chen B, McClements DJ. Improving the Efficacy of Essential Oils as Antimicrobials in Foods: Mechanisms of Action. Annu Rev Food Sci Technol. 2019 Mar 25;10:365-387.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999 May;26(9-10):1231-7.

Roberto, D.; Micucci, P.; Sebastian, T.; Graciela, F.; Anesini, C. Antioxidant activity of limoneneon normal murine lymphocytes: Relation to H2O2 modulation and cell proliferation. Basic Clin.Pharmacol. Toxicol. 2010, 106, 38–44

Routray W, Orsat V. “Plant By-Products and Food Industry Waste: A Sourceof Nutraceuticals and Biopolymers,” In: Grumezescu M, Holban AM, editors.Handbook of Food Bioengineering, Food Bioconversion. (2017). 279–315.

Sahaa A, Basaka BB. Scope of value addition and utilization of residualbiomass from medicinal and aromatic plants. Ind Crops Prod. (2020) 145:111979.

Salamon I, Ibraliu A , Fejer J, (2014) Essential oil of common juniper (Juniperus communis L.) In Albania. Conference paper : Proceedings of the Eighth Conference on Medicinal and Aromatic Plants of Southeast European Countries (8th CMAPSEEC) 19-22 May 2014, Durrës, Albania, pp.239-244.

Sela, F.; Karapandzova, M.; Stefkov, G.; Cvetkovikj, I.; Trajkovska-Dokik, E.; Kaftandzieva, A.; Kulevanova, S. Chemical composition and antimicrobial activity of leaves essential oil of Juniperus communis (Cupressaceae) grown in Republic of Macedonia. Maced. Pharm. Bull. 2013, 59, 23–32.

Shamir F, Ahmadi L, Mirza M, Korori SAA. Secretoryelements of needles and berries of Juniperuscommunis L. ssp. communis and its volatileconstituents. Flavour Frag J 2003; 18:425-428.

Van den Dool H and Kratz PD, (1963) A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. Journal of Chromatography 11, 463.

Wang Q, Rehman M, Peng D, Liu L. Antioxidant capacity and a-glucosidaseinhibitory activity of leaf extracts from ten ramie cultivars. Ind Crop Prod. (2018) 122:430–7.

BIOPOLYMER-BASED BIODEGRADABLE PACKAGING MATERIALS FOR FOOD APPLICATIONS

Arzu AKPINAR BAYIZIT[1,2], *Burcu CAM*[3*], *Yagmur Eda OZBEK*[3]

[1]Faculty of Agriculture, Department of Food Engineering, Bursa Uludag University, 16059 Bursa, Türkiye.
[2]Bursa Uludag University, Science and Technology Application and Research Center, 16059, Bursa, Türkiye.
[3]Bursa Uludag University, Graduate School of Natural and Applied Sciences, 16059 Bursa, Türkiye.

Corresponding Author: burcucam3@gmail.com

ABSTRACT

Biopolymer-based materials are considered an alternative to petroleum-based plastics, which are responsible for much of the environmental pollution in food packaging. Biopolymers can be produced by microorganisms or derived from biomass. Starch, cellulose, chitin, chitosan, polylactic acid (PLA), and polyhydroxylalkanoates (PHAs) are biopolymers that are produced according to origin and method of production. These materials are used to produce biodegradable packaging, and these are preferred for sustainability, waste reduction and non-toxicity properties. However, high price, lower mechanical, thermal, and barrier properties against oxygen, water vapor, microbes, light, and conditions of high humidity compared to petroleum-based polymers limit their use in the food industry. Studies on biodegradable polymers coupled with various materials are expected to play an increasingly crucial role to improve these properties that restrict their industrial use. Ultimately, continued advancements and innovations in biopolymer technology holds promise for overcoming these challenges and fostering wider adoption of sustainable packaging solutions in the food industry. Here we review the benefits, chemistry and trends in biopolymer-based packaging materials for food applications.

Keywords: Biopolymer-based packaging materials, biodegradable packaging, biopolymers, sustainability

INTRODUCTION

Food packaging has a crucial role in the food industry to ensure the quality and safety of the food, as it helps not only to contain and carry goods from one place to another, but also to preserve, protect, present, market, and communicate with its mechanical and barrier properties (Ojha et al., 2015; Verma et al., 2021; Saha, 2023). Over a period, the packaging technology has evolved more to improve the shelf life of the product with ensured quality, using both modern technology and conventional methods.

Paper, plastic, metal, and glass are the materials used for packaging applications. Glass and metal, having high barrier properties, display insignificant interaction with the containment, whereas conventional petroleum-based plastics are preferred on the grounds of low cost, durability, resistance to degradation, high versatility, flexibility, transparency and heat sealability (Table 1) (Licciardello, 2017; Awulachew, 2022; Cheng et al., 2024). However, about 95-99% of plastics have been made from non-renewable sources and food packaging accounts for 50% of the plastics that have been made (Ncube et al., 2020; Shaikh et al., 2021). Food packaging plastics have half-lives ranging from 2 to more than 2500 years in the environment and that creates serious pollution (Amin et al., 2022) and approximately 70% of marine litter objects are single-use plastic items such as straws or food and drink containers (Omerović et al., 2021).

Table 1. Differences between plastics and bioplastics

Conventional plastics	Bioplastics
Produced from fossil fuels and petrochemicals	Produced from natural resources
Limited and nonsustainable resources	Sustainable and renewable resources
Almost all are non-biodegradable	Most are biodegradable
High mechanical strength, could withstand heavy load	Low mechanical strength
Low water permeability	High water permeability
High thermal stability	Low thermal stability
Low brittleness	High brittleness
Takes hundred years to degrade into smaller particles	Under controlled composting conditions biodegrades within 3–6 months
Eco-toxic and causes environmental pollution	Eco-friendly
High greenhouse gas emission	Emits fewer greenhouse gases and
Increases global warming	Considered as carbon neutral
More energy consumption during production	Less energy usage during production
Reduces soil fertility	Increases soil fertility
Technically mature production steps	Early stage of development, needs more study on production and recycling process

Since the food supply is expected to increase in parallel to the increase in the world's population, the demand for food packaging would also increase. The growing use of packaging materials and hurdles for natural recycling operations coupled with high costs had driven the need for renewable food packaging materials that have efficient physical, mechanical, and barrier properties (Ncube et al., 2020).

The emphasis is on developing packaging materials that are compatible with food products, eco-friendly, and lightweight for reducing the amount of material use, waste and as well as transportation costs. Therefore, plastics from biopolymers, known as biodegradable materials, are promising alternatives to fulfil this requirement as being compatible with foods and having sustainable properties to bring down the environmental impacts of food packaging. However, their use is mostly restricted due to high price and lower mechanical, thermal, and barrier properties against oxygen, water vapor, microorganisms, light, and conditions of high humidity. In order to improve these negativeness they mostly are combined with nanoparticles (Mekonnen et al. 2013; Jafarzadeh & Jafari, 2020; Wrona & Nerín, 2020; Zhao et al., 2020; Shaikh et al., 2021; Visco et al., 2022; Ghasemlou et al., 2024).

Biodegradable Packaging

Bioplastics are the materials derived directly from natural polymers with or without modifications, produced by microbial fermentations, and chemically synthesized from renewable raw materials, such as proteins, wood, potatoes, corn, vegetable oils, and food waste (Figure 1) (Abe et al., 2021; Xia et al., 2021; Keränen et al., 2022; Behera et al., 2022). In scientific literature two terms are described for bioplastics: bio-based and biodegradable. “Biobased” plastics are the materials that are wholly or partly substituted by renewable biomass, whereas “biodegradable” refers to materials that can naturally disintegrated or decomposted by microorganisms, resulting in carbon dioxide, water, and biomass (Ghasemi-Mobarakeh et al., 2019; Bhagwat et al., 2020; Bachleitner et al., 2023; Zhao et al., 2024).

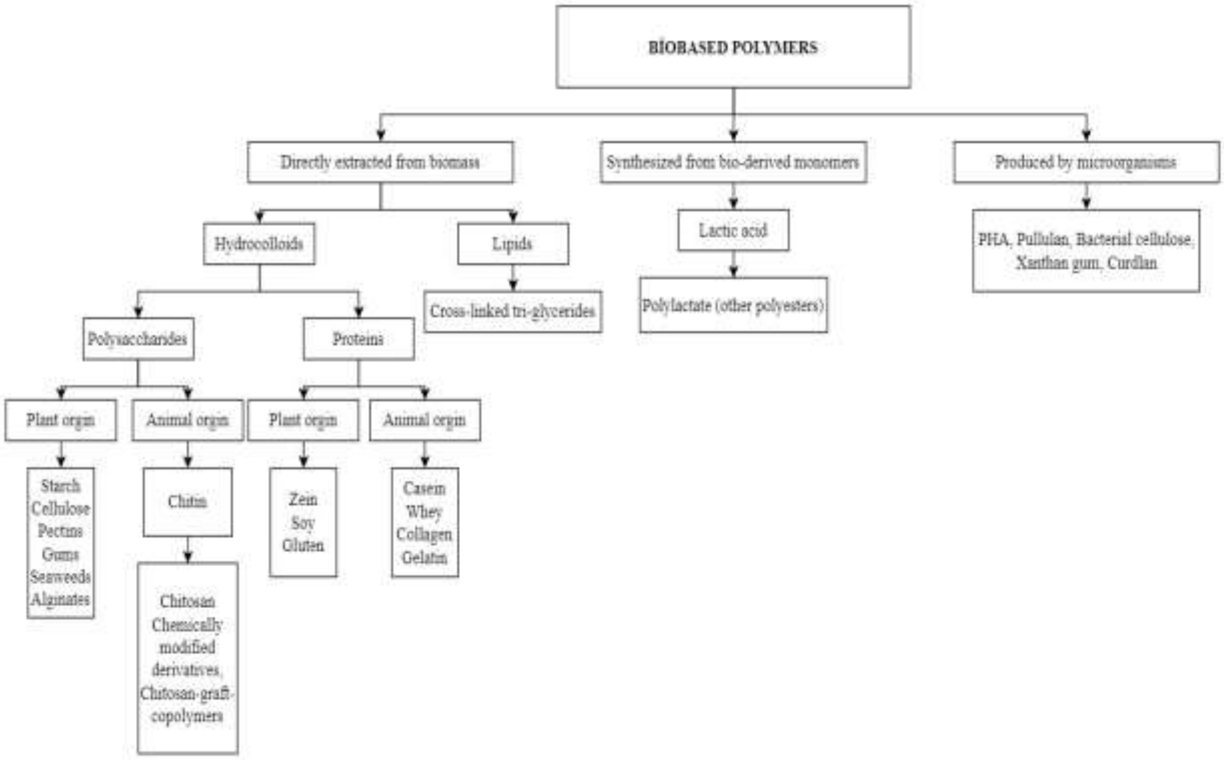


Figure 1. Types of biopolymers used in packaging

For a substance to be considered as "biodegradable" some studies find composting within 1-2 months is sufficient (Amin et al., 2022; Cheng et al., 2024); however, as stated by International Standard Organization (ISO), any substance could be considered as biodegradable if its initial mass is reduced by 90% after six months under compost conditions of 50^0C (ISO, 2018). Furthermore, The American Standard for Testing Materials (ASTM) accepts a composting rate of 60% for homopolymers and 90% for blends within 180 days (ASTM, 2024).
Biodegradable polymers at present only replace about 1% of the global plastic production, but its market is growing rapidly (Shaikh et al., 2021). In 2023, the global production capacities of biodegradable/biobased packaging materials were estimated as 2.18 million tonnes, which is forecasted to reach approximately 7.43 million tonnes in 2028.

Materials of Biodegradable Packaging

Naturally sourced biopolymers are the first choice when attempting to achieve a less toxic and more sustainable food packaging because of their biocompatibility, biodegradability, and nontoxicity (Omerović et al., 2021). Biodegradable plastics are the degradable plastics in which the degradation results from the action of living microorganisms such as bacteria, fungi, and algae into water, carbon dioxide, and biomass without the release of any toxic byproducts (Zhao et al., 2020). It is worth mentioning neither all bio-based plastics are biodegradable, nor all biodegradable plastics are bio-based. The biodegradable plastics comprised of bioplastics, whose building components are derived from renewable raw materials, and other plastics manufactured from the petrochemicals possess biodegradable additives. Thus, according to their degradation degree biodegradable plastics can be grouped as completely degradable or non-degradable (semi-degradable) (Ivanković et al., n.d.; Shaikh et al., 2021; Cheng et al., 2024).

It is important to note that non-degradable bioplastics are advantageous than biodegradable fossil fuel plastics. They have better recyclability, compatibility and low levels of carbon dioxide production than existing plastic processing systems. Starch, cellulose, chitin, chitosan, polylactic acid (PLA), polyhydroxyalkanoate (PHA), polyhydroxybutyrate (PHB), and polycaprolactone (PCL) are the mostly employed biopolymers for production of bottles, jars, cans, buckets, food containers, disposable cups, packaging films, and refuse bags (Pawar & Purwar, 2013; Jabeen et al., 2015; Nesic et al., 2020; Ncube et al., 2020).

Table 2. A summary of the types, form, applications and key features of starch and cellulose-based bioplastics from recent literature

Type of Polymer	Form of Packaging	Application	Key Features	References
Starch	Film	To improve bilayer food packaging films; PPS (Pure potato starch) and 3-APTMS (3-(Aminopropyl) Trimethoxy silane) crosslinked to make PPS-3APTMS films. These bilayer films were then made by solution casting the PPS-3APTMS with PLA.	Crosslinking reduced the hydrophilicity. PPS-3APTMS-PLA bilayer films improved thermal stability and mechanical properties. Tensile strength increased. Solubility, swelling ratio, water vapor permeability and transparency decreased. Crosslinked PPS-3APTMS bilayer film has 5.45% biodegradation. PPS-3APTMS-PLA bilayer film has 5.08% biodegradation.	(Cheng et al., 2024)

Chitosan/ Potato Starch	Film	Chitosan (CS)/ Potato Starch (PS) films cross-linked with citric acid (CA) at different concentration (5%-10%-15%-20%).	15% CA showed best comprehensive properties all among all films.Improved water resistance properties,	(Wu et al., 2019)
Cassava starch/ Chitosan	Active packaging	Cassava starch (CS) /chitosan (CH) film were combined with pitanga (Eugenia uniflora L.) leaf extract (PE) and/or natamycin (NA) in different formulations. (2.25 g PE/100g FFS, 1g NA/100g FFS, 2.25g PE+1g NA/100g FFS)	The addition of PE didn't affect mechanical properties, whereas NA significantly decreased the flexibility of films. While only the PE added film showed significant antioxidant activity, only NA added film showed antifungal effect against *Aspergillus flavus* and *Aspergillus parasiticus*.	(Sirisha Nallan Chakravartula et al., 2020)
Chitosan/ Starch	Active edible film	Chitosan/Starch film incorporated with nano titanium dioxide (TiO2-N) and clove oil (CO) in different concentrations.	TiO2-N improved the compactness of the film, increased the tensile strength (TS) and antioxidant activity, and decreased the water vapour permeability (WVP). CO decreased TS but increased the hydrophobicity, water vapour barrier antimicrobial and antioxidant properties.	(Li et al., 2019)
PLA/ Starch	Film	PLA/starch blends were loaded with cellulose nanofibers, cellulose nanocrystals and aqueous extract from coffee husk.	Adding extract from coffee husk didn't improve mechanical properties but reduced oxygen permability and showed antioxidant activity.	(Collazo-Bigliardi et al., 2019)

Cellulose/ Chitosan	Film	Prepared different 6 solution of composition of Cellulose/Chitosan and N-methylmorpholine-N-oxide (NMMO).	The tensile strength is the highest and has the smoothest surface with the solution containing 3% chitosan but at 5% chitosan reduces notably because of phase separation.	(Shih et al., 2009)
Hemicelluloses/ Chitosan	Edible film	Hemicelluloses/Chitosan films loaded with 5% cellulose nanofibers using different plasticizers. (10-20-30-40wt%/ glycerol, sorbitol, xylitol)	The film plasticized with sorbitol (20wt%) showed the highest tensile strength and glycerol (20 wt%) showed the highest tensile strain at break.	(Xu et al., 2019)
Bacterial cellulose/ Poly(3-hydroxybutyrate)	Active packaging	Bacterial cellulose (BC) were produced from *Gluconacetobacter hansenii,* Poly(3-hydroxybutyrate) (PHB) and clove essential oil (CLO) blended.	It was observed that adding 10wt% clove oil caused a 65% microbial reduction.	(Albuquerque et al., 2021)
Chitosan/ Cellulose acetate phthalate /Nano ZnO	Film	Chitosan (CS) /Cellulose acetate phthalate (CAP) loaded with nano ZnO.	The film loaded with 5 wt% ZnO nano particles showed low surface wettability, high tensile strength, and thermal and barrier properties compared to chitosan/ cellulose acetate phthalate films.	(Indumathi et al., 2019)
Corn Starch/ Chitosan	Active packaging	To see properties of different concentrations of Chitosan in active packaging, Corn starch (CS) and chitosan solutions in different chitosan	Increasing chitosan concentration increased film solubility, color change, tensile strength, and elongation at break, decreased water vapor permeability.	(Ren et al., 2017)

		concentrations (21, 41, 61, 81% of starch weight) blended.	Elongation at break peaked at 41% chitosan which decreased as concentration increased.	
Chitosan	Active packaging	The researchers prepared composite films by incorporating chitosan (CS) and polypyrrole(PPy) into microfibrillated cellulose (MFC).	Adding PPy increased surface uniformity and filled pores in the composite films. Antimicrobial and antioxidant activity increased. The MFC/CS/PPy composite films showed good thermal stability. The oxygen transmission rate was crucially reduced.	(Gao et al., 2020)
PBAT/TPS	Functional active packaging	PBAT (polybutylene adipate-co-terephthalate)/TPS polymer blended with ZnO nanoparticles (1-5%) for meat packaging.	PBAT/TPS films containing 4% ZnO showed superior microbial controls for total viable count, lactic acid bacteria and yeast and mold. The shelf life of meat packaged in PBAT/TPS films with 3% ZnO was extended by more than 3 days.	(Phothisarattana et al., 2022)
OP/LLDPE	Film	LLDPE/OP masterbatch pellets were produced and combined with neat LLDPE using specific processing parameters determined to produce blown LLDPE/OP composite films differing in OP	OP enhanced the UV and visible light blocking capacity. Composite films had reduced tensile strength and elongation at break. LLDPE/OP films with up to 11.5% OP had shown water vapor barrier	(Fehlberg et al., 2023)

		content (5, 10, 11.5, and 12.5%).	properties, crystallinity, and melting temperature compared to neat LLDPE films.	
PLA/PHB	Film	PLA-PHB blend films with 75 wt% PLA and 25 wt% PHB prepared and ternary blends with 15 wt % d-limonene added.	Improved oxygen barrier properties, surface water resistance, and thermal stability. The addition of d-limonene improved flexibility and elongation at break; improved processabilityIt decreased the glass transition temperature.	(Arrieta et al., 2014)

Starch and Celluloses

Starch, the main storage polysaccharide in the vegetal cells, is made of linear amylose and branched amylopectin molecules, of which have α-D-(l,6)-glucose monomers, and is insoluble in water and alcohol. When starch is gelatinized it loses its original conformation and forms a melted gel similar to synthetic thermoplastics (Gadhave et al., 2018; Ghasemlou et al., 2020, 2022). Starch is a key ingredient considered as a biodegradable polymer material for its availability, low cost, biodegradability, renewability, and eco-friendliness. Since starch is obtained from green plants, such as potatoes, corn, wheat and rice, due to its nontoxic nature it ensures safety in food, pharmaceuticals, and consumer products when it gets into direct contact (Henning et al., 2022; Hossain et al., 2024).
Starch is mostly regarded as an alternative to synthetic polymers as thermoplastic starch (TPS). TPS is highly sensitive to humidity and the thermal properties changes with the content of water. The amylose/amylopectin ratio of starch significantly impacts the structure and thermal stability of its bioplastics. The elongation and strength increase as the amylose content increases. Corn-starch is generally preferred as TPS for food packaging applications (Abe et al., 2021; Shaikh et al., 2021; Thulasisingh et al., 2022).
However, pure starch-based bioplastics are too brittle and fragile, and as a result of higher rate of crystallinity, strong intermolecular forces, and the presence of disordered granules their processability and mechanical strength is reduced. Thus, plasticization of starch, either by plasticizer effect of water or plasticizers, is the common way to improve its flexibility, and allow extrusion (Rao et al., 2022; Ghasemlou et al., 2024). Moreover, starch-based materials are prone to moisture sensitivity, which can lead to degradation

and microbial growth. To optimize the overall properties and amplify the processability and functional properties combining starch with aliphatic polyesters such as Polyglycolide (PGA), Polylactide (PLA), and Polycaprolactone (PCL) have been recommended (Encalada et al., 2018; Surendren et al., 2022). Blending starches with such before mentioned materials aims to reduce the production cost; to improve barrier properties and dimensional stability; to decrease the hydrophilic character of starch; and increase its biodegradability.

Further, starch derivatives, cellulose esters, cellulose acetate, nitrocellulose, and celluloid are available biodegradable polymers for packaging (Verma et al., 2021). Cellulose is composed of complex carbohydrates. It is a linear polysaccharide that depends on the type and treatment of the raw materials. Cellulosic polymeric materials are mostly available as bacterial cellulose, microcrystalline cellulose, cellulose nanocrystals or nano whiskers.

Cellulose-based bioplastics are sustainable, have barrier properties, work with current infrastructure, and break down over time. Cellulose properties encompass low density, high durability, non-toxicity, biocompatibility, biodegradability, transparency and good film-forming characteristics with exceptional mechanical, thermal and barrier properties, making cellulose as an ideal packaging material (Thulasisingh et al., 2022).

Regenerated cellulose film (RCF) is one of the first packaging films obtained from pure cellulose fiber derived from woods. Modified cellulose and different cellulose derivatives such as cellulose acetate and cellulose esters are predominant materials used in industry for molding, extrusion and film applications. However, have poor solubility and indigestibility. Cellulose can be transformed to various forms with its insolubility and low fluency (Shaikh et al., 2021), however, lacks antibacterial activity therefore it needs to blend with antimicrobial agents (Motelica et al., 2020). The naturally derived, high molecular weight cellulose can be made flexible by plasticizing with humectants (glycol-type compounds) (Saha et al., 2022).

Chitin and Chitosan

Crystalline chitin has low immunogenicity, biocompatibility, antimicrobial activity, environment-friendliness and biodegradability, which makes it highly attractive substrate for the biomaterial world. Chitin is the most abundant aminopolysaccharide polymer occurring in nature composed mainly of (1-4)-linked 2-acetamido-2-deoxy-β-D-glucose monomers. It is the main component of exoscheleton from crustacean such as crab, and shrimp and insects and cell walls of fungi and yeasts. Chitin acts as a structural material, similar to the role cellulose in plants forming crystalline nanofibrils or whiskers. This cellulose-like biopolymer is distinguished from other polisaccharides by its nitrogen content due to the replacement of glucose with N-acetylglucosamine. This allows increased hydrogen bonding between adjacent monomers, resulting in increased strength for the chitin-polymer matrix (Robertson, 2014; Azizati and Haqiki, 2021; Fuhrmann and Zuberer, 2021; Chen et al., 2023).

Chitosan is an approved food ingredient, however, it has limited industrial applicability due to low mechanical and thermal stability, as well as high sensitivity to water (Kumari et al., 2022). Therefore, chitosan derivatives to improve solubility or biodegradability have been produced to introduce new functions or properties. Chitosan oligomers are soluble over a wide pH range, from acidic to basic, compared to chitosan only being soluble in acidic aqueous media. Lack of solubility at neutral and basic pH has hindered the use of chitosan in some applications. Chitosan is the N-deacetylated derivative of chitin. This natural polysaccharide is produced from deacetylation by enzymatic preparations or treatment with hot alkali (Elieh-Ali-Komi and Hamblin, 2017; Ivanković et al., 2017; Azizati and Haqiqi, 2021; Lárez-Velásquez, 2023). As in the same manner of chitin the structure of chitosan is similar to cellulose except the glucose at position C-2 being replaced by acetamido and/or amino groups (Dutta et al., 2004; Rinaudo, 2006; Srinivasa and Tharanathan, 2007; Priyadarshi and Rhim, 2020). Owing to the specific intermolecular hydrogen bonding network of chitin, its solubility in water is very difficult. However, as chitosan being in deacetylated form can be readily dissolved in dilute acidic aqueous solutions.

The solubility, viscosity, film forming capacity, nontoxicity, bio-compostability, biodegradability, ion binding and antimicrobial properties make chitosan a multipurpose polymer for various food applications, including food packaging (Wang et al., 2018; Crini, 2022; Thulasisingh et al., 2022). The most they use as an edible coating to prolong the shelf- life of fresh fruits and vegetables Chitosan has very poor mechanical properties and resistance to water. Both chitin and chitosan and their derivatives exert biological activities such as antitumoral, antimicrobial, antioxidant, and anti-inflammatory activities, that designates them as therapeutic polymers. Chitin and chitosan showed good antimicrobial properties for various fungi, yeasts, and bacteria found in foods (Ivanković et al., 2017; Shaikh et al., 2021). Its film forming properties and biological activity invite new applications. Being soluble in aqueous solutions, this pseudonatural cationic polymer is largely used in different applications as solutions, gels, or films and fibers (Kaisler et al., 2020; Aranaz et al., 2021).

Polylactic acid (PLA)

Polylactic acid (PLA) is one of the most signified thermoplastic biopolymers made up of renewable resources, lactic acid which is produced either chemically through microbial fermentation or synthetically through the hydrolysis of lactonitrile. Mostly lactides and lactic acid monomers are depolymerized to give PLA. PLA has been acknowledged as safe for all industrial uses involving food packaging. It has been categorized as GRAS (generally recognized as safe) as the migration of LA from PLA packaging containers to food is insignificant (Scarfato et al., 2015; Castro-Aguirre et al., 2016; Nath et al., 2023; Ghasemlou et al., 2024).

In contrast to other biopolymers, PLA has garnered a greater degree of interest in the packaging industry because the existing technology utilized for conventional plastics can be employed for its processing without any modifications required. It can be processed by injection molding, thermoforming, film extrusion, and blow molding. PLA can be plasticized with monomers or oligomeric lactic acid (Aliotta et al., 2019; Mangaraj et al., 2019; Kumari et al., 2022). Its worth mentioning that it also has comparable mechanical characteristics with conventional polymers including PET, PS, and polycarbonate (PC). It is an eco-friendly biodegradable thermoplastic polyester since it slowly degrades over a period of months to two years whilst petroleum based plastics take more.

Polylactic acid has many desirable properties such as high transparency, easy fabrication, biocompatibility, biodegradability, recyclability, superior mechanical strength, non-toxicity and better thermal plasticity. Its main problem is its weak barrier property and oxygen can easily contact it (Motelica et al., 2020) and thermal resistance and high brittleness restrict its applications (Brdlík et al., 2021).

PLA is suitable for fresh foods and those that don't need protection from oxygen in the food packaging industry, it may also be used in food trays, bottles, candy wrappers, and cups. Also because of its high-water permeability, it is ideal for various packaging uses, including extending the shelf life of fresh fruit and bread (Nath et al., 2023). PLA products are preferred as mostly single-use primary packaging plastics such as cups, bottles, clam shells and wrappers for ready-to-eat meals. These packages have short service lifetime and easily find their way into the environment subsequently being thrown away after consumption. Hence their degradability is an important phenomenon to control environment pollution (Karamanlioglu et al. 2017; Imam et al., 2019; Silva et al., 2019; Ncube et al., 2020).

Polyhydroxyalkanoates (PHAs)

PHAs are a family of aliphatic polyesters generated through bacterial fermentations. A range of microorganisms can form and accumulate PHAs as storage materials when cultivated under low and unbalanced nutrient conditions. The production procedures of PHAs include microbial fermentation processes followed by extraction of the metabolite. The carbon source, microbial strain, and pretreatments are effective on the final physical and mechanical properties of PHAs obtained. They are recognized as nontoxic, nonsticky, and insoluble in water (Ghasemlou et al., 2024).

PHAs can be categorized according to their chain length as short-chain (3-5 carbon atoms), medium-chain (6-14 carbon atoms), and long-chain (15 or more carbon atoms) (Westlie et al., 2022). Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH) are the two most well-known PHAs.

In addition to their biodegradable, biocompatible, and renewable nature, PHAs possess properties such as good ultra-violet resistance, high tensile strength, printability, grease and oil resistance, which make them ideal for use in the food packaging industry (Kumari et al., 2022). PHAs have thermo-mechanical properties similar to synthetic polymers such as polypropylene (Mangaraj et al., 2019). The soft and amorphous form of PHAs offers

better flexibility than crystalline or semi-crystalline forms of PHA, which are brittle (González-López et al., 2023; Ladhari et al., 2023).
PHAs exhibit diverse properties depending on the specific monomers incorporated during biosynthesis, offering a wide range of potential applications in food packaging. PHAs such as polyhydroxybutyrate (PHB) possess similar mechanical properties as PE, but their lamellar structure provides higher aroma barrier properties which are useful for short-term food packaging. Poly-3-hydroxybutyrate has comparable characteristics to some commonly used polyolefins such as polyethylene terephthalate, high-density polyethylene, low-density polyethylene and polypropylene in terms of high melting temperature, tensile strength, high resistance to ultraviolet radiation, glass transition temperature and crystallinity (Sirohi et al. 2021a,b).
PHA polymers can be molded into soft films or strong plastics by conventional processing methods to produce cups, jars, trays, or single-use packaging utensils (Rekhi et al., 2022), besides bottles, containers, sheets, films, laminates, fibers and coatings (Mangaraj et al., 2019). The PHA market share is less than 2% within all bioplastics, on the grounds of high production cost for large-scale up applications, almost ten times more than conventional plastics. Use of recombinant technology or mixed cultures, along with improvements in fermentation, recovery, and purification processes might help PHA commercialization. However, high brittleness, low impact strength and low melt viscosity are the other major hurdles which have negative impact on the technological feasibility of PHAs (Torabi et al., 2023), which might be reduced by the use of plasticizers, copolymerization, and blending with other polymers or the addition of inorganic or organic fillers.

Conclusion and Future Work

Bioplastics are sustainable, biodegrable and cost-effective polymers that exhibit comparable properties to conventional plastics with less environmental pollution impact. The waste management and recycling process of fossil-based plastics is hard and complex. Moreover, single-use plastics in food packaging industry display a significant environmental problem. Here bioplastics are considered as a potential solution to reduce the environmental threat for waste collection and recycling. However, biopolymers used for food packaging lack some technical, economic, and legislative deficiencies. Hence, to shift from conventional plastics to bio-based products more research and development is needed in processing and recycling techniques, mechanical properties, large-scale production, cost, durability, sustainability, greenhouse gas emissions and optimum biodegradation.

REFERENCES

Abe, M.M., Martins, J.R., Sanvezzo, P.B., Macedo, J.V., Branciforti, M.C., Halley, P., Botaro, V.R., & Brienzo, M. (2021). Advantages and Disadvantages of Bioplastics

Production from Starch and Lignocellulosic Components. Polymers, 13(15), 2484. https://doi.org/10.3390/polym13152484

Albuquerque, R.M.B., Meira, H.M., Silva, I.D.L., Silva, C.J.G., Almeida, F.C.G., Amorim, J.D.P., Vinhas, G.M., Costa, A.F.S., & Sarubbo, L.A. (2021). Production of a Bacterial Cellulose/Poly(3-Hydroxybutyrate) Blend Activated with Clove Essential Oil for Food Packaging. Polymers and Polymer Composites, 29(4), 259–270. https://doi.org/10.1177/0967391120912098

Aliotta, L., Gigante, V., Coltelli, M.B., Cinelli, P., & Lazzeri, A. (2019). Evaluation of Mechanical and Interfacial Properties of Bio-Composites Based on Poly (Lactic Acid) with Natural Cellulose Fibers. International Journal of Molecular Science, 20, 960. https://doi.org/10.3390/ijms20040960

Amin, U., Khan, M.K.I., Maan, A.A., Nazir, A., Riaz, S., Khan, M.U., Sultan, M., Munekata, P.E.S., & Lorenzo, J.M. (2022). Biodegradable Active, Intelligent, and Smart Packaging Materials for Food Applications. Food Packaging and Shelf Life, 33, 100903. https://doi.org/10.1016/j.fpsl.2022.100903

Amin, U., Khan, M. U., Majeed, Y., Rebezov, M., Khayrullin, M., Bobkova, E., Shariati, M. A., Chung, I. M., & Thiruvengadam, M. (2021). Potentials of polysaccharides, lipids and proteins in biodegradable food packaging applications. International Journal of Biological Macromolecules, 183, 2184–2198.

Anonymous. (2023). Bioplastics Market Development Update. https://docs.european-bioplastics.org/publications/market_data/2023/EUBP_Market_Data_Report_2023.pdf

Aranaz, I., Alcántara, A. R., Civera, M. C., Arias, C., Elorza, B., Heras Caballero, A., & Acosta, N. (2021). Chitosan: An Overview of Its Properties and Applications. Polymers, 13(19), 3256. https://doi.org/10.3390/polym13193256

Arrieta, M. P., López, J., Hernández, A., & Rayón, E. (2014). Ternary PLA-PHB-Limonene Blends Intended for Biodegradable Food Packaging Applications. European Polymer Journal, 50(1), 255–270.

ASTM D883-17. (2024). Standard Terminology Relating to Plastics. https://www.astm.org/d0883-17.html

Awulachew, M.T. (2022). A Review of Food Packaging Materials and Active Packaging System. International Journal of Health Policy and Planning, 1(1), 28–35.

Azizati, Z., Haqiqi, A.K. (2021). Synthesis and Characterization of Bioplastic Based on Chitosan-Urea-Bentonite. Young Scholar Symposium on Science Education and Environment (YSSSEE), IOP Publishing, 1796, 012108. https://doi.org/10.1088/1742-6596/1796/1/012108

Bhargava, N., Sharanagat, V. S., Mor, R. S., & Kumar, K. (2020). Active and Intelligent Biodegradable Packaging Films Using Food and Food Waste-Derived Bioactive Compounds: A Review. Trends in Food Science and Technology, 105, 385–401.

Brdlík, P., Borůvka, M., Běhálek, L., & Lenfeld, P. (2021). Biodegradation of Poly(Lactic Acid) Biocomposites under Controlled Composting Conditions and Freshwater Biotope. Polymers, 13(4), 1–15.

Chakravartula, S.N., Lourenço, R.V., Balestra, F., Quinta Barbosa Bittante, A.M., Sobral, P. J. do A., & Dalla Rosa, M. (2020). Influence of Pitanga (*Eugenia uniflora* L.) Leaf Extract and/or Natamycin on Properties of Cassava Starch/Chitosan Active Films. Food Packaging and Shelf Life, 24, 100498. https://doi.org/10.1016/j.fpsl.2020.100498

Chen, W., Li, Y., Tan, Q., Xie, L., Hou, Y., Jiang, W., Peipei, D., Wu, H., Liu, H., Chen, Z., Mao, C., & Farooq, T.H. (2023). Biowaste Valorisation in a Circular Economy. In Advances in Pollution Research: Recent Trends in Solid Waste Management, Eds. Ravindran, B., Gupta, S.K., Bhat, S.A., Chauhan, P.S., Tyagi, N., Elsevier, pp. 245–258.

Cheng, J., Gao, R., Zhu, Y., & Lin, Q. (2024). Applications of Biodegradable Materials in Food Packaging: A Review. Alexandria Engineering Journal, 91, 70–83.

Collazo-Bigliardi, S., Ortega-Toro, R., & Chiralt, A. (2019). Using Lignocellulosic Fractions of Coffee Husk to Improve Properties of Compatibilised Starch-PLA Blend Films. Food Packaging and Shelf Life, 22. https://doi.org/10.1016/j.fpsl.2019.100423

Crini, G. (2022). Chitin and Chitosan: Production, Properties, and Applications. In Chitin and Chitosan: Discoveries and Applications for Sustainability, by Crini, G., Academic Press, pp. 147–202.

Demarteau, J., Cousineau, B., Wang, Z., Bose, B., Cheong, S., Lan, G., Baral, N.R., Teat, S.J., Scown, C.D., & Keasling, J.D. (2023) Biorenewable and Circular Polydiketoenamine Plastics. Nature Sustainability, 6(11), 1426–1435.

Elieh-Ali-Komi, D., & Hamblin, M.R. (2016). Chitin and Chitosan: Production and Application of Versatile Biomedical Nanomaterials. International Journal of Advanced Research, 4(3), 411–427.

Encalada, K., Aldás, M.B., Proaño, E., & Valle, V. (2018). An Overview of Starch-Based Biopolymers and their Biodegradability. Ciencia e Ingeniería, 39(3), 245–263.

Fehlberg, J., McKay, S., Matuana, L.M., & Almenar, E. (2023). Use of Orange Juice Processing Waste to Produce Films using Blown Film Extrusion for Food Packaging. Journal of Food Engineering, 341. https://doi.org/10.1016/j.jfoodeng.2022.111337

Fuhrmann, J.J., & Zuberer, D.A. (2021). Carbon Transformations and Soil Organic Matter Formation. In Principles and Applications of Soil Microbiology (Third Edition), Eds. Gentry, T.J., Fuhrmann, J.J., & Zuberer, D.A., Elsevier, pp. 327–361.

Gadhave, R., Das, A., Mahanwar, P., & Gadekar, P. (2018) Starch Based Bio-Plastics: The Future of Sustainable Packaging. Open Journal of Polymer Chemistry, 8, 21–33.

Gao, Q., Lei, M., Zhou, K., Liu, X., Wang, S., & Li, H. (2020). Preparation of a Microfibrillated Cellulose/Chitosan/Polypyrrole Film for Active Food Packaging. Progress in Organic Coatings, 149. https://doi.org/10.1016/j.porgcoat.2020.105907

GBT 20197-2006 (2024). Define, Classify, Marking and Degradability Requirement of Degradable Plastic.

http://openstd.samr.gov.cn/bzgk/gb/newGbInfo?hcno=97FCB8AB9135BC8B636 25 3071C1AA6C2

Ge, C., Lansing, B., & Lewis, C.L. (2021). Thermoplastic Starch and Poly(Vinyl Alcohol) Blends Centered Barrier Film for Food Packaging Applications. Food Packaging and Shelf Life, 27, 100610. https://doi.org/10.1016/j.fpsl.2020.100610

Ghasemlou, M., Daver, F., Ivanova, E.P., Murdoch, B.J., & Adhikari, B. (2020). Use of Synergistic Interactions to Fabricate Transparent and Mechanically Robust Nanohybrids Based on Starch, Non-Isocyanate Polyurethanes, and Cellulose Nanocrystals. ACS Applied Materials & Interfaces, 12(42), 47865–47878.

Ghasemlou, M., Daver, F., Murdoch, B.J., Ball, A.S., Ivanova, E.P., & Adhikari, B. (2022). Biodegradation of Novel Bioplastics Made of Starch, Polyhydroxyurethanes and Cellulose Nanocrystals in Soil Environment. Science of the Total Environment, 815, 152684.

Ghasemlou, M., Barrow, C.J., & Adhikari, B. 2024. The Future of Bioplastics in Food Packaging: An Industrial Perspective. Food Packaging and Shelf Life, 43, 101279. https://doi.org/10.1016/j.fpsl.2024.101279

Henning, F.G., Ito, V.C., Demiate, I.M., & Lacerda, L.G. (2022). Non-conventional Starches for Biodegradable Films: A Review Focussing on Characterisation and Recent Applications in Food Packaging. Carbohydrate Polymer Technologies and Applications, 4, 100157. https://doi.org/10.1016/j.carpta.2021.100157

Hossain, Md. T., Shahid, Md. A., Akter, S., Ferdous, J., Afroz, K., Refat, K.R.I., Faruk, O., Jamal, M.S.I., Uddin, Md. N., & Samad, Md. A.B. (2024). Cellulose and Starch-Based Bioplastics: A Review of Advances and Challenges for Sustainability. Polymer-Plastics Technology and Materials, 63(10), 1329–1349.

Imam, S., Glenn, G., & Chiellini, E. (2012). Utilization of Biobased Polymers in Food Packaging: Assessment of Materials, Production and Commercialization. In Emerging Food Packaging Technologies: Principles and Practice, Eds. Yam K.L., & Lee, D.S., Woodhead Publishing Series in Food Science, Technology and Nutrition, pp. 435–468.

Indumathi, M. P., Saral Sarojini, K., & Rajarajeswari, G. R. (2019). Antimicrobial and Biodegradable Chitosan/Cellulose Acetate Phthalate/Zno Nano Composite Films with Optimal Oxygen Permeability and Hydrophobicity for Extending the Shelf Life of Black Grape Fruits. International Journal of Biological Macromolecules, 132, 1112–1120. https://doi.org/10.1016/j.ijbiomac.2019.03.171

ISO 2018 ISO 14855–2:2018 (2018) Determination of The Ultimate Aerobic Biodegradability of Plastic Materials under Controlled Composting Conditions – Method by Analysis of Evolved Carbon Dioxide – Part 2: Gravimetric Measurement of Carbon Dioxide Evolved in a Laboratory-Scale Test. International Organization for Standardization. ISO Central Secretariat, Geneva, Switzerland.

Ivanković, A., Zeljko, K., Talić, S., Bevanda, A.M., & Lasić, M. (2017). Biodegradable Packaging in the Food Industry. Journal of Food Safety and Food Quality, 68(2), 26–38.

Jabeen, N., Majid, I., & Nayik, G.A. (2015). Bioplastics and Food Packaging: A Review. Cogent Food & Agriculture, 1, 1117749. https://doi.org/10.1080/23311932.2015.1117749

Jafarzadeh, S., & Jafari, S.M. (2020). Impact of Metal Nanoparticles on the Mechanical, Barrier, Optical and Thermal Properties of Biodegradable Food Packaging Materials. Critical Reviews in Food Science and Nutrition, 61(16), 1–19.

Kaisler, M., van den Broek, L.A.M. & Boeriu, C.G. (2020). Chitin and Chitosan as Sources of Bio-Based Building Blocks and Chemicals. In Chitin and Chitosan: Properties and Applications, Eds. van den Broek, L.A.M., Boeriu, C.G., Stevens, C.V., Wiley, pp 229–244.

Karamanlioglu, M., Preziosi, R., & Robson, G.D. (2017). Abiotic and Biotic Environmental Degradation of the Bioplastic Polymer Poly(Lactic Acid): A Review. Polymer Degradation and Stability, 137, 122–130.

Kumari, S.V.G., Pakshirajan, K., & Pugazhenthi, G. (2022). Recent Advances and Future Prospects of Cellulose, Starch, Chitosan, Polylactic Acid and Polyhydroxyalkanoates for Sustainable Food Packaging Applications. International Journal of Biological Macromolecules, 221, 163–182.

Ladhari, S., Vu, N.-N., Boisvert, C., Saidi, A., & Nguyen-Tri, P. (2023). Recent Development of Polyhydroxyalkanoates (PHA)-based Materials for Antibacterial Applications: A Review. ACS Applied Bio Materials, 6(4), 1398–1430.

Lárez-Velásquez, C. (2023). Chitosan: An Overview of Its Multiple Advantages Forcreating Sustainable Development Poles. Polímeros, 33(1), e20230005. https://doi.org/10.1590/0104-1428.20220103

Li, W., Zheng, K., Chen, H., Feng, S., Wang, W., & Qin, C. (2019). Influence of Nano Titanium Dioxide and Clove Oil on Chitosan-Starch Film Characteristics. Polymers, 11(9). https://doi.org/10.3390/polym11091418

Licciardello, F. (2017). Packaging, Blessing in Disguise. Review on Its Diverse Contribution to Food Sustainability. Trends in Food Science and Technology, 65, 32–39.

Mangaraj, S., Yadav, A., Bal, L. M., Dash, S. K., & Mahanti, N. K. (2019). Application of Biodegradable Polymers in Food Packaging Industry: A Comprehensive Review. Journal of Packaging Technology and Research, 3(1), 77–96.

Mekonnen, T., Mussone, P., Khalil, H., Bressler, D. (2013). Progress in Bio-Based Plastics and Plasticizing Modifications. Journal of Materials Chemistry A, 1,13379–13398.

Motelica, L., Ficai, D., Ficai, A., Oprea, O. C., Kaya, D. A., & Andronescu, E. (2020). Biodegradable Antimicrobial Food Packaging: Trends and Perspectives. Foods-MDPI Basel, 9(10), 1438. https://doi.org/10.3390/foods9101438

Nanda, S., Patra, B. R., Patel, R., Bakos, J., & Dalai, A. K. (2022). Innovations in Applications and Prospects of Bioplastics and Biopolymers: A Review. Environmental Chemistry Letters, 20(1), 379–395.

Nath, P. C., Sharma, R., Debnath, S., Sharma, M., Inbaraj, B. S., Dikkala, P. K., Nayak, P. K., & Sridhar, K. (2023). Recent Trends in Polysaccharide-Based Biodegradable Polymers for Smart Food Packaging Industry. International Journal of Biological Macromolecules, 253(8), 127524. https://doi.org/10.1016/j.ijbiomac.2023.127524

Ncube, L.K., Ude, A.U., Ogunmuyiwa, E.N., Zulkifli, R., & Beas, I.N. (2020). Environmental Impact of Food Packaging Materials: A Review of Contemporary Development from Conventional Plastics to Polylactic Acid Based Materials. Materials-MDPI Basel, 13(21), 4994. https://doi.org/10.3390/ma13214994

Nesic, A., Castillo, C., Castaño, P., Barjas, G.C., & Serrano, J. (2020). Bio-Based Packaging Materials. In Biobased Products and Industries, Ed. Galanakis, C.M., Elsevier: Amsterdam, The Netherlands, pp. 279–309.

Ojha, A., Sharma, A., Sihag, M., & Ojha, S. (2015). Food Packaging Materials and Sustainability – A Review. Agricultural Reviews, 36(3), 241–245.

Omerović, N., Djisalov, M., Živojević, K., Mladenović, M., Vunduk, J., Milenković, I., Knežević, N., Gadjanski, I., & Vidić, J. (2021). Antimicrobial Nanoparticles and Biodegradable Polymer Composites for Active Food Packaging Applications. Comprehensive Reviews in Food Science and Food Safety, 20(3), 2428–2454.

Pawar, P., & Purwar, A.H. (2013). Biodegradable Polymers in Food Packaging. American Journal of Engineering Research, 2, 151–164.

Phothisarattana, D., Wongphan, P., Promhuad, K., Promsorn, J., & Harnkarnsujarit, N. (2022). Blown Film Extrusion of PBAT/TPS/Zno Nanocomposites for Shelf-Life Extension of Meat Packaging. Colloids and Surfaces B: Biointerfaces, 214, 112472. https://doi.org/10.1016/j.colsurfb.2022.112472

Priyadarshi, R., & Rhim, J.-W. (2020). Chitosan-Based Biodegradable Functional Films for Food Packaging Applications. Innovative Food Science & Emerging Technologies, 62, 102346. https://doi.org/10.1016/j.ifset.2020.10234

Rao, L. S., Naidu, C., & Tiwari, S. (2022). Investigation on Synthesis, Structure and Degradability of Starch Based Bioplastics. Materials Today: Proceedings, 49, 257–261.

Ren, L., Yan, X., Zhou, J., Tong, J., & Su, X. (2017). Influence of Chitosan Concentration on Mechanical and Barrier Properties of Corn Starch/Chitosan Films. International Journal of Biological Macromolecules, 105, 1636–1643.

Rinaudo, M. (2006). Chitin and Chitosan: Properties and Applications. Progress in Polymer Science, 31, 603–632.

Robertson, G.L. (2014). Food Packaging. In Reference Module in Food Science: Encyclopedia of Agriculture and Food Systems, Ed. Van Alfen, N.K., Academic Press, pp. 232–249.

Saha, N.C. (2022). Food Packaging: Concepts and Its Significance. In Food Packaging Materials, Techniques and Environmental Issues, Eds. Saha, N.C., Ghosh, A.K., Garg, M., Sadhu, S.D., Springer Nature, Switzerland AG, pp. 1–46.

Scarfato, P., Di Maio, L., & Incarnato, L. (2015). Recent Advances and Migration Issues in Biodegradable Polymers from Renewable Sources for Food Packaging. Journal of Applied Polymer Science, 132, 13. https://doi.org/10.1002/app.42597

Shih, C.M., Shieh, Y.T., & Twu, Y.K. (2009). Preparation and Characterization of Cellulose/Chitosan Blend Films. Carbohydrate Polymers, 78(1), 169–174.

Shaikh, S., Yaqoob, M., & Aggarwal, P. (2021). An Overview of Biodegradable Packaging in Food Industry. Current Research in Food Science, 4, 503–520.

Silva, T.F.D., Menezes, F., Montagna, L.S., Lemes, A.P., & Passador, F.R. (2019). Effect of Lignin as Accelerator of the Biodegradation Process of Poly(Lactic Acid)/Lignin Composites. Material Science and Engineering B, 251, 114441. https://doi.org/10.1016/j.mseb.2019.114441

Sirohi, R., Gaur, V.K., Pandey, A.K., Sim, S.J., & Kumar, S. (2021). Harnessing Fruit Waste for Poly-3-Hydroxybutyrate Production: A Review. Bioresource Technology, 326, 124734. https://doi.org/10.1016/j.eti.2020.101271

Sirohi, R., Pandey, J.P., Tarafdar, A., Agarwal, A., Chaudhuri, S.K., & Sindhu, R. (2021). An Environmentally Sustainable Green Process for the Utilization of Damaged Wheat Grains for Poly-3-Hydroxybutyrate Production. Environmental Technology Innovations, 21, 101271. https://doi.org/10.1016/j.biortech.2021.124734.

Srinivasa, P.C., & Tharanathan, R.N. (2007). Chitin/Chitosan – Safe, Ecofriendly Packaging Materials with Multiple Potential Uses. Food Reviews International, 23(1), 53–72.

Swain, P.K., Das, P., & Pal, V. (2023). Production of Eco-friendly Plastics and Bioplastics from Crustacean Shells and Their Environmental Applications. In Novel Polymeric Materials for Environmental Applications, Eds. Das, P., & Das, S., World Publishing (WSPC): India, pp 233-261.

Surendren, A., Mohanty, A.K., Liuc, Q., & Misra, M. (2022). A Review of Biodegradable Thermoplastic Starches, their Blends and Composites: Recent Developments and Opportunities for Single-Use Plastic Packaging Alternatives. Green Chemistry, 24, 8606–8636.

Thulasisingh, A., Kumar, K., Yamunadevi, B., Poojitha, N., SuhailMadharHanif, S., & Kannaiyan, S. (2022). Biodegradable Packaging Materials. Polymer Bulletin, 79(7), 4467–4496.

Torabi, H., McGreal, H., Zarrin, H., & Behzadfar, E. (2023). Effects of Rheological Properties on 3D Printing of Poly (lactic acid)(PLA) and Poly (hydroxy alkenoate)(PHA) Hybrid Materials. ACS Applied Polymer Materials, 5(6), 4034–4044.

Verma, M.K., Shakya, S., Kumar, P., Madhavi, J., Murugaiyan, J., & Rao, M.V.R. (2021). Trends in Packaging Material for Food Products: Historical Background, Current Scenario, and Future Prospects. Journal of Food Science and Technology, 58, 4069–4082.

Visco, A., Scolaro, C., Facchin, M., Brahimi, S., Belhamdi, H., Gatto, V., & Beghetto, V. (2022). Agri-Food Wastes for Bioplastics: European Prospective on Possible

Applications in their Second Life for a Circular Economy. Polymers-MDPI Basel, 14, 2752. doi: 10.3390/polym14132752

Westlie, A.H., Quinn, E.C., Parker, C.R., & Chen, E.Y.-X. (2022). Synthetic Biodegradable Polyhydroxyalkanoates (Phas): Recent Advances and Future Challenges. Progress in Polymer Science, 134, 101608. https://doi.org/10.1016/j.progpolymsci.2022.101608

Xu, J., Xia, R., Zheng, L., Yuan, T., & Sun, R. (2019). Plasticized Hemicelluloses/Chitosan-Based Edible Films Reinforced by Cellulose Nanofiber with Enhanced Mechanical Properties. Carbohydrate Polymers, 224, 115164. https://doi.org/10.1016/j.carbpol.2019.115164

Zhao, L., Rong, L., Zhao, L., Yang, J., Wang, L., & Sun, H. (2020). Plastics of the Future? The Impact of Biodegradable Polymers on the Environment. In Microplastics in Terrestrial Environments – Emerging Contaminants and Major Challenges, Eds. He, D., Luo, Y., Springer Nature, Switzerland AG. https://doi.org/10.1007/698_2020_462

Zhao, X., Wang, Y., Chen, X., Yu, X., Li, W., Zhang, S., Meng, X., Zhao, Z.-M., Dong, T., Anderson, A., Aiyedun, A., Li, Y., Webb, E., Wu, Z., Kunc, V., Ragauskas, A., Ozcan, S., & Zhu, H. (2023). Sustainable bioplastics derived from renewable natural resources for food packaging, Matter, 6(1), 97–127.

SURFACE ANALYSIS OF ULTRAFILTRATION MEMBRANES

Ayşe Seda APAYDIN[1], Pelin ONSEKİZOĞLU BAĞCI[1]

[1] Trakya University, Engineering Faculty, Food Engineering Department, Edirne, Turkey
Corresponding author e-mail: asedaapaydin@trakya.edu.tr

ABSTRACT

In ultrafiltration processes, membrane surface properties are one of the critical factors that directly affect filtration performance. Membrane fouling can be evaluated depending on the morphology, chemical composition and electrical charge of the membrane used. Furthermore, membrane modifications affect fouling tendencies and play a crucial role in membrane selection. Determination of membrane surface features contributes to the optimization of the ultrafiltration process. In this study, an investigation on membrane fouling characterization, membrane modifications and fouling trends was carried out focusing on surface analysis of ultrafiltration membranes. Various surface characterization techniques such as contact angle method, FTIR-ATR, SEM, AFM and surface load analysis are discussed and how these techniques determine membrane surface properties and their effects on fouling mechanisms are presented.

Keywords: Ultrafiltration membranes, Surface analysis, Membrane fouling

INTRODUCTION

In the food industry, ultrafiltration (UF) technology is used for concentration, clarification and separation of macromolecular solutions (Castro-Muñoz, Boczkaj, Gontarek, Cassano & Fíla, 2020). During UF, macro- and micro-components in the feed fluid accumulate in the porous structure or on the surface of the polymeric membrane, causing membrane fouling. This phenomenon brings many negative consequences.

Surface features of membranes like hydrophilicity/hydrophobicity, surface roughness and charge significantly affect fouling behavior (Xiao, Wang, Huang, Waite & Wen, 2011). Hydrophilic surfaces generally tend to be less fouled, while hydrophobic surfaces tend to be more fouled. Surface roughness affects the adsorption capacity of foulants, and rougher surfaces are generally subject to more fouling. Furthermore, surface load can also determine fouling behavior through electrostatic interactions (Breite, Went, Prager & Schulze, 2015). Various methods are used to analyze the fouling layer consisted on the surface of membranes. In this context, the use of contact angle, FTIR-ATR, SEM, AFM and surface load analysis for the characterization of membrane fouling in ultrafiltration processes was mentioned and membrane surface properties were examined.

CHARACTERIZATION OF ULTRAFILTRATION MEMBRANE SURFACES

Contact Angle Method

The contact angle method is used to identify the degree of hydrophilicity /hydrophobicity of the membrane surface. These measurements are made using a contact angle measuring device. Contact angle meters analyze the angle formed by a liquid drop with the membrane surface, using an optical system (Hebbar, Isloor & Ismail, 2017). In ultrafiltration technique, the surface properties of membranes significantly affect their processing performance. The tendency of the membrane surface to absorb or repel water can be determined by contact angle measurements. Generally, lower contact angles were observed in PES membranes with hydrophilic modifications, while high contact angles were observed in hydrophobic membranes (Otitoju, Ahmad & Ooi, 2018). As a result, it can be said that hydrophilic surfaces interact better with water and reduce fouling tendencies. As shown in Figure 1, it is stated that the membrane has a hydrophilic surface when the contact angle is smaller than 90˚, a hydrophobic surface when it is greater than 90˚, and a super hydrophobic surface above 150˚ (Yürekli, Polatoğlu & Baştürk, 2015). In the studies, it has been observed that lower contact angle values are obtained when the pore size of the membrane is increased (Susanto & Ulbricht, 2005). In summary, the contact angle method is important in terms of evaluating the effectiveness of modifications applied to membrane surfaces.

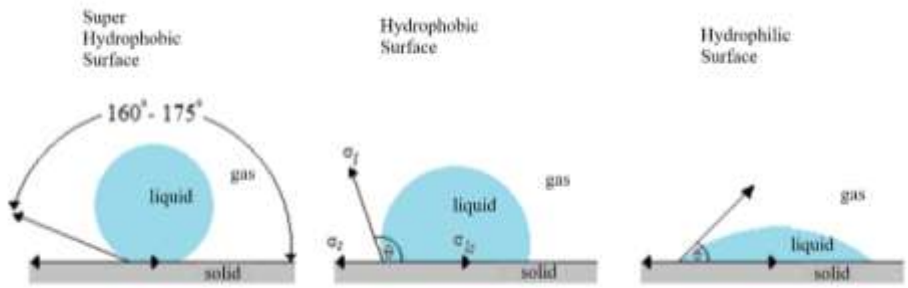


Figure 1. Contact angle results of different membrane surfaces (Yürekli, et al., 2015).

FTIR-ATR Analysis

Fourier Transform Infrared (FT-IR) spectroscopy and Attenuated Total Reflectance (ATR) method are used to analyze the chemical composition of fouling, and the layers formed on the membrane surface (Liu, Chen & Yu, 2020). The principle of the analysis is based on the reflection of infrared light sent to the sample surface (Glassford, Byrne & Kazarian, 2013), and the interaction between the ATR crystal and the surface shows how much the functional groups on the membrane surface absorb IR light. The ATR crystal is able to detect changes on the surface with a thickness of less than 1 micrometer (Yürekli, Polatoğlu & Baştürk, 2015). Phenolic compounds or other organic substances in the feed liquid used in ultrafiltration can be identified by this method. It has been reported that FT-IR spectra help to determine the functional groups and chemical bonds of organic and inorganic foulants (Gaffney, Marley & Jones, 2002). In a study investigating the chemical composition of clean and dirty UF membranes, the polymer structures of the clean membrane were first identified, so that the contaminant particles after the filtration process could be identified more easily with the help of the peaks obtained (Oldani & Schock, 1989). In a study investigating the surface characterization of membranes, it was observed that new bonds were formed in the FTIR spectra of modified membranes. It was reported that membranes with stronger properties were obtained with the bonds formed (Biederman, et al., 2001). As a result of all these, it is seen that FTIR-ATR analysis contributes to the optimization of the filtration process by determining the surface modifications and fouling mechanisms of membranes.

SEM Analysis

Scanning Electron Microscopy (SEM) is used to analyze the morphology and roughness of the membrane surface by imaging the surface topography in three dimensions at high resolution (Zhong, Li, Zhang & Xing, 2012). In SEM analysis, an electron beam is sent onto the sample, where it interacts with the electrons released on the surface. With the help of a detector, the electrons are collected and various images are created (Joy & Joy, 1996). It has been stated that the cross-sectional image obtained for ultrafiltration membranes provides information about the variation of porosity along the thickness, density and thickness of the upper layer otherwise, surface images provide information about membrane pore size and density (Yürekli, Polatoğlu & Baştürk, 2015). In various SEM analyses, it was observed that PES membranes with hydrophilic modification have smoother surfaces and less accumulation on the membrane surface. Conversely, the surfaces of membranes with hydrophobic character are rougher and more prone to fouling (Ahmad, Abdulkarim, Ooi & Ismail, 2013). In a study examining the surface properties of membranes with different polymeric structures, it was reported that more porous, ups and downs images were obtained in the membrane with a more hydrophilic structure (Gulec, Bagci & Bagci, 2017). Thus, SEM analysis helps to determine the fouling mechanisms by examining the morphological properties of foulants deposited on the membrane area.

AFM Analysis

Atomic Force Microscopy (AFM) is used to idetify surface roughness and nanometric structures on the surface (Russell, Batchelor & Thornton, 2001). AFM allows to obtain the topography of the surface of a sample by scanning over its surface with a very fine tip (probe). This probe moves up and down very close to the surface of the sample and the movements are measured with the help of a sensor (Sun, 2018). In this way, a detailed map of the membrane surface at the nanometer scale can be obtained. In ultrafiltration membranes, AFM is used to evaluate the surface roughness and to determine the size and shape of the pores on the area (Boussu, et al., 2005). In a study where a three-dimensional picture of the membrane surface was taken and the shadows were colored, it was reported that the higher areas were lighter and the lower areas were darker (Hilal, & Johnson, 2010). Surface roughness is an important factor in membrane fouling, and rougher surfaces can increase membrane fouling by facilitating the adhesion and deposition of particles. It has also been observed that the adhesion force of the AFM probe to the membrane area can determine the hydrophilic or hydrophobic properties of the surface (Brant & Childress, 2004). In studies, it is stated that hydrophilic surfaces generally have higher surface energy and reduce membrane fouling by causing water to spread more easily on the surface (Díez & Rosal, 2020). In addition, it has been observed that the cake layers formed on the surface can be examined in more detail using AFM in studies using UF (Li, et al., 2018). In summary, AFM is important in understanding the surface properties of ultrafiltration membranes and investigating the effects of these properties on membrane fouling.

Surface Load Analysis

Surface load analysis is used to specify the electrical charge of the membrane surface. To determine this electrical charge, zeta potential measurements are required (Nghiem, Schäfer & Elimelech, 2006). Zeta potential refers to the electrical potential difference in the double layer structure around a surface and varies depending on factors such as ionic strength, pH and chemical composition of the membrane surface (Attard, Antelmi & Larson, 2000). Surface load can be determined by zeta potential device. The device calculates the surface charge as zeta potential and shows the results graphically. In ultrafiltration membranes, the surface load can take positive, negative or neutral values depending on the chemical composition of the membrane material and surface modifications. It is stated that surfaces with high zeta potential generally reduce membrane fouling by repelling particles with the same charge, but this may alter depending on the fouling mechanism (Kochkodan, Johnson & Hilal, 2014). It has also been observed that in membranes with a more hydrophilic structure, foulants with the same charge electrostatically repel each other and the zeta potential takes negative values (Breite, Went, Prager & Schulze, 2015). Therefore, selecting a membrane surface that is appropriately loaded according to the nature of the foulant particles in the feed fluid has been reported to be an substantial factor in reducing membrane fouling (Kumar & Ismail, 2015). In a study showing that membrane fouling largely depends on the zeta potential and hydrophilicity of the surface, it was stated that membrane fouling can be avoided by changes in pH values and electrostatic repulsion forces (Breite, Went, Prager & Schulze, 2015). Thus, zeta potential helps to evaluate membrane fouling behavior by determining the electrostatic interactions between membrane and foulants and the charge density on the surface of particles.

CONCLUSIONS

The surface chemistry, morphology and charge properties of membranes directly affect their fouling behavior and filtration performance during the ultrafiltration process. The mentioned analysis methods ensure information about the chemical composition, physical structure and interactions of membrane fouling with the surface. The contact angle method determines the hydrophilic or hydrophobic nature of the membrane, while FT-IR and ATR analyze the chemical structure of the foulants. SEM and AFM visualize the morphology and surface roughness of the fouling layer. Zeta potential measurements determine the electrostatic interactions between the foulants and the membrane. The combination of these methods allows for a comprehensive characterization of membrane fouling. In this way, it may be possible to develop fouling prevention strategies and increase the efficiency of membrane systems.

REFERENCES

Ahmad, A. L., Abdulkarim, A. A., Ooi, B. S., & Ismail, S. (2013). Recent development in additives modifications of polyethersulfone membrane for flux enhancement. Chemical engineering journal, 223, 246-267.

Attard, P., Antelmi, D., & Larson, I. (2000). Comparison of the zeta potential with the diffuse layer potential from charge titration. *Langmuir*, *16*(4), 1542-1552.

Biederman, H., Boyaci, I. H., Bilkova, P., Slavinska, D., Mutlu, S., Zemek, J., ... & Mutlu, M. (2001). Characterization of glow-discharge–treated cellulose acetate membrane surfaces for single-layer enzyme electrode studies. Journal of applied polymer science, 81(6), 1341-1352.

Boussu, K., Van der Bruggen, B., Volodin, A., Snauwaert, J., Van Haesendonck, C., & Vandecasteele, C. (2005). Roughness and hydrophobicity studies of nanofiltration membranes using different modes of AFM. Journal of colloid and interface science, 286(2), 632-638.

Brant, J. A., & Childress, A. E. (2004). Colloidal adhesion to hydrophilic membrane surfaces. Journal of Membrane Science, 241(2), 235-248.

Breite, D., Went, M., Prager, A., & Schulze, A. (2015). Tailoring membrane surface charges: A novel study on electrostatic interactions during membrane fouling. Polymers, 7(10), 2017-2030.

Castro-Muñoz, R., Boczkaj, G., Gontarek, E., Cassano, A., & Fíla, V. (2020). Membrane technologies assisting plant-based and agro-food by-products processing: A comprehensive review. Trends in Food Science & Technology, 95, 219-232.

Díez, B., & Rosal, R. (2020). A critical review of membrane modification techniques for fouling and biofouling control in pressure-driven membrane processes. Nanotechnology for Environmental Engineering, 5(2), 15.

Gaffney, J. S., Marley, N. A., & Jones, D. E. (2002). Fourier transform infrared (FTIR) spectroscopy. Characterization of materials, 1-33.

Glassford, S. E., Byrne, B., & Kazarian, S. G. (2013). Recent applications of ATR FTIR spectroscopy and imaging to proteins. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1834(12), 2849-2858.

Gulec, H. A., Bagci, P. O., & Bagci, U. (2017). Clarification of apple juice using polymeric ultrafiltration membranes: A comparative evaluation of membrane fouling and juice quality. Food and bioprocess technology, 10, 875-885.

Hebbar, R. S., Isloor, A. M., & Ismail, A. F. (2017). Contact angle measurements. In Membrane characterization (pp. 219-255). Elsevier.

Hilal, N., & Johnson, D. (2010). The use of atomic force microscopy in membrane characterization. In Basic aspects of membrane science and engineering (pp. 337-354). Elsevier Inc..

Joy, D. C., & Joy, C. S. (1996). Low voltage scanning electron microscopy. Micron, 27(3-4), 247-263.

Kochkodan, V., Johnson, D. J., & Hilal, N. (2014). Polymeric membranes: Surface modification for minimizing (bio) colloidal fouling. *Advances in colloid and interface science*, *206*, 116-140.

Kumar, R., & Ismail, A. F. (2015). Fouling control on microfiltration/ultrafiltration membranes: Effects of morphology, hydrophilicity, and charge. *Journal of Applied Polymer Science*, *132*(21).

Li, W., Ling, G., Lei, F., Li, N., Peng, W., Li, K., ... & Zhang, Y. (2018). Ceramic membrane fouling and cleaning during ultrafiltration of limed sugarcane juice. Separation and Purification Technology, 190, 9-24.

Liu, X. Y., Chen, W., & Yu, H. Q. (2020). Probing protein-induced membrane fouling with in-situ attenuated total reflectance fourier transform infrared spectroscopy and multivariate curve resolution-alternating least squares. Water Research, 183, 116052.

Nghiem, L. D., Schäfer, A. I., & Elimelech, M. (2006). Role of electrostatic interactions in the retention of pharmaceutically active contaminants by a loose nanofiltration membrane. Journal of Membrane Science, 286(1-2), 52-59.

Oldani, M., & Schock, G. (1989). Characterization of ultrafiltration membranes by infrared spectroscopy, ESCA, and contact angle measurements. Journal of membrane science, 43(2-3), 243-258.

Otitoju, T. A., Ahmad, A. L., & Ooi, B. S. (2018). Recent advances in hydrophilic modification and performance of polyethersulfone (PES) membrane via additive blending. RSC advances, 8(40), 22710-22728.

Russell, P., Batchelor, D., & Thornton, J. (2001). SEM and AFM: complementary techniques for high resolution surface investigations. Veeco Instruments Inc., AN46, Rev A, 1, 2004.

Sun, W. (2018). Principles of atomic force microscopy. Atomic Force Microscopy in Molecular and Cell Biology, 1-28.

Susanto, H., & Ulbricht, M. (2005). Influence of ultrafiltration membrane characteristics on adsorptive fouling with dextrans. Journal of membrane science, 266(1-2), 132-142.

Xiao, K., Wang, X., Huang, X., Waite, T. D., & Wen, X. (2011). Combined effect of membrane and foulant hydrophobicity and surface charge on adsorptive fouling during microfiltration. Journal of Membrane Science, 373(1-2), 140-151.

Yürekli, Y., Polatoğlu, İ., & Baştürk, S. B. (2015). Filtrasyon Membranların Üretim ve Karakterizasyon Yöntemleri. Celal Bayar Üniversitesi Fen Bilimleri Dergisi, 11(2), 175-181.

Zhong, Z., Li, D., Zhang, B., & Xing, W. (2012). Membrane surface roughness characterization and its influence on ultrafine particle adhesion. Separation and purification technology, 90, 140-146.

FUNCTIONAL FOODS AND THEIR EFFECTS ON HEALTH

Kübra Demir, Orhan Onur AŞKIN

Kırklareli University

ABSTRACT

In recent years, there has been a significant increase in interest in natural products as individuals have become more health-conscious and aware of disease prevention. People are turning to functional foods to protect and improve their health, rather than relying on chemically-based medications. These foods are preferred more frequently due to their nutritional properties and their role in preventing diseases.

Functional foods can be defined as products that have positive effects on health thanks to certain biologically active components added to them. For example, adding ingredients such as omega-3 fatty acids, probiotics, fibers, and antioxidants to foods makes these products functional. These types of foods offer various benefits, such as strengthening the immune system, protecting heart health, ensuring proper digestive function, and generally enhancing quality of life by supporting specific metabolic processes in the body.

Another important feature of functional foods is that they do not contain synthetic ingredients. These foods can be included in a daily diet, consumed directly, or found in the form of food and beverages. While they serve as a part of the daily diet due to their nutritional qualities, they also stand out for their functions in reducing the risk of diseases and improving overall health. For this reason, functional foods occupy an important place in modern nutritional approaches.

In conclusion, functional foods play a significant role in supporting individual health and are increasingly finding a place in today's dietary habits. In an era where lifestyle diseases are on the rise, the consumption of such foods has great potential to protect individual health and improve public health levels.

Keywords: Functional foods, health benefits, nutraceuticals.

INTRODUCTION

Foods contain the nutrients required for an individual's survival, normal development, and growth. Recent advancements in food and nutrition science have demonstrated that foods not only meet the nutritional needs of individuals but also play a role in regulating various body functions and preventing certain diseases [1].

Foods have three primary functions: nutritional, sensory, and physiological. While nutritional and sensory functions are found in every food, only some foods possess physiological functions. However, with various technological processes applied in recent years, it has become possible to endow foods with physiological functions [1].

Functional foods are those that provide additional benefits beyond meeting the body's basic nutritional needs by exerting positive effects on human physiology and metabolic functions. As such, they play a role in disease prevention and promoting a healthier lifestyle. Functional foods can be natural, unprocessed food items, or they can be enriched with functional components or modified through genetic engineering methods to be consumed as part of the daily diet [2].

For food to be considered functional, it must contain active agents such as bioactive compounds, probiotic microorganisms, and prebiotic substances, and these agents must be adequately delivered to the relevant areas of the body. The effect of a bioactive compound should not be confused with the alleviation of symptoms caused by its deficiency; it should be based on the benefit it provides beyond its primary function [3].

Recent advancements in food science and technology have also led to the emergence of new terminologies such as novel foods, nutraceuticals, designer foods, pharmacofoods, and phytosceuticals. Nutraceuticals refer to both traditional and different forms of food components (such as tablets, capsules, etc.), while functional foods denote traditional food forms. Examples of functional foods include probiotic and prebiotic yogurts, margarines enriched with olive oil, various herbal teas and seeds (such as green tea, walnuts, flaxseed, etc.), certain seafood (rich in essential fatty acids), meat products enriched with fiber, antioxidant-rich foods, and low-cholesterol eggs [4].

Probiotics/Prebiotics

Probiotics are defined as live microbial food additives that provide beneficial effects on health by residing in the digestive system [5]. Probiotics contribute to the intestinal microbial balance by enhancing the flora, binding to receptors via competition to leave no space for pathogens, and facilitating their excretion through feces. Microorganisms used as probiotics include Lactobacillus acidophilus, GG, rhamnosum, lactis, Streptococcus thermophilus, and yeasts such as cerevisia and inigeroryze [6].

Prebiotics are non-digestible food ingredients that promote the growth of beneficial colon bacteria, which positively affect human and animal health. The most commonly known prebiotic substances are oligosaccharides. Oligosaccharides are non-digestible polysaccharides consisting of 3-10 sugar units linked by glycosidic bonds. As prebiotics, oligosaccharides have the ability to bind to fimbriae, which allow pathogenic microorganisms to adhere to the intestinal surface, thereby preventing colonization of pathogenic microorganisms and facilitating their excretion through feces [7].

Foods can be made functional with probiotics and/or prebiotics. Because bacteria such as lactic acid used in the production of various fermented dairy products like yogurt cannot survive in the digestive system, probiotics like Lactobacillus acidophilus and bifidobacteria are added to these products. In recent years, these bacteria have been sold in tablet form, as capsules, and by freeze-drying methods in the market. The presence of both prebiotics and probiotics in the same product as synbiotics enables the utilization of the beneficial functional effects of both when consumed [7].

The microflora in the digestive system plays a very important role in the health of humans and animals. The effects of lactobacilli on human health were first proposed by Metchnikoff at the beginning of this century. Probiotic bacteria are known to have antibacterial activities against pathogenic bacteria, and they have many positive effects on the immune system and anti-allergic responses [5]. According to the World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO), probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.

The consumption of probiotic-containing products has beneficial effects on health, strengthening gut and body health, and combating other diseases, and the benefits of these bacteria to human health have been known for a long time. The most important of these benefits is the regulation of the gastrointestinal system. However, they also contribute to the prevention and improvement of many other diseases. For example, probiotics are reported to have therapeutic applications such as preventing hypercholesterolemia, protecting against colon/bladder cancer, preventing osteoporosis, protecting against diarrhea, alleviating constipation, and preventing urogenital infections. Additionally, they have benefits like enhancing the immune system, protecting the normal intestinal microflora against pathogens, and lowering blood pressure [8].

Prebiotics, as fermentable carbohydrate sources for intestinal bacteria, play a significant role in reducing the risks of colon cancer by producing short-chain fatty acids and suppressing toxicogenic bacterial reactions. They lower intestinal pH, thereby increasing the solubility and absorption of minerals like calcium, magnesium, and iron ions. Moreover, the interaction between calcium and short-chain fatty acids also enhances mineral absorption. Long-term consumption reduces the risk of osteoporosis by increasing bone density. When they cause the intestinal environment to become acidic, they reduce the activity of procarcinogenic enzymes (7α-hydroxylase and nitroreductase). They are particularly effective in relieving intestinal problems like constipation in elderly individuals where Bifidobacterium species in the intestinal flora have decreased and been replaced by harmful bacteria. Prebiotics are effective in preventing food allergies in infants by increasing the number of probiotics. When prebiotics are used in conjunction with probiotic Bifidobacterium species, physiological and health effects are observed. Prebiotics promote the growth of bifidobacteria and are therefore important in manifesting the health benefits of bifidobacteria. Additionally, prebiotics inhibit the proliferation of pathogenic bacteria, have a laxative effect, reduce the risk of developing diarrhea, lower serum triglyceride levels, and reduce postprandial glucose and insulin levels in animal studies. Oligosaccharides themselves act like cell surface receptor analogs, binding pathogenic microorganisms and facilitating their excretion through feces [9].

Antioxidants

The primary sources of antioxidant substances are fruits and vegetables, which are widely consumed in daily diets. These foods contain essential nutrients such as water, various vitamins, minerals, polyphenolic compounds, and dietary fiber, and they are low in salt, making them one of the most important food groups in human nutrition. Recent epidemiological studies have shown that fruits and vegetables play a significant role in maintaining health and preventing diseases. High consumption of fruits and vegetables has been reported to help reduce the risk of chronic diseases such as cataracts, chronic lung diseases, hypertension, cardiovascular diseases, Alzheimer's, Parkinson's, and cancer. These benefits are attributed to the antioxidant components found in fruits and vegetables. Substances with antioxidant functions include vitamins C, E, and A, carotenoids, flavonoids, organic acids, melanoidins, and phenolic compounds. The antioxidant effects of especially vitamins E, C, and carotenoids have been demonstrated in numerous studies [10].

The primary effect of antioxidants on human health is their role as free radical scavengers. During metabolic activities, oxygen can be converted into reactive oxygen species, such as superoxide, hydrogen peroxide, singlet oxygen, and hydroxyl radicals, making oxygen highly toxic to living systems. In the presence of antioxidants, oxidative stress-related damage is significantly reduced in the body. Antioxidants act as hydrogen atom donors, converting chain-forming radicals into less reactive species. They prevent lipid peroxidation, cross-linking of proteins, and DNA mutations. Antioxidants neutralize oxidants through four different mechanisms:

- **Scavenging effect**: This occurs by converting oxidants into weaker molecules.
- **Quenching effect**: This involves neutralizing oxidant substances by transferring a hydrogen atom, primarily done by flavonoids.
- **Repair effect**: Antioxidants exert this effect by eliminating damage caused by oxidants.
- **Chain-breaking effect**: This effect, performed by hemoglobin and vitamin E, involves binding to oxidants and inhibiting their function [11].

Following the discovery of the beneficial effects of antioxidants, synthetic antioxidants were produced, but their use was associated with some side effects. This led to an increased preference for natural antioxidants. In one study, egg yolk, which is high in antioxidant content, was added to meatballs made from turkey meat. It was found that increasing the amount of egg yolk added to the meatballs correspondingly increased the antioxidant capacity [12].

Ice Creams

Ice cream is generally a dairy product enjoyed by people of all ages, made by processing a mixture (ice cream mix) of milk and dairy products (such as milk powder, cream), sweeteners, stabilizers, emulsifiers, colorants, and flavoring agents with added air in freezers [13].

Studies on functional dairy products hold an important place within research on functional food products. Due to their health benefits and their presence in almost every individual's daily diet, milk and dairy products are highly suitable for transformation into functional products. Functional ice cream is also an important component among functional dairy products. Besides its health benefits, the ease of modifying its composition and its popularity among people of all ages have increased research into making ice cream functional.

Ice cream is a high-nutrition product widely produced and consumed in almost all countries due to its significant nutritional content. In our country, it is mainly known as a dairy product consumed for pleasure, especially during hot summer days to cool off. Ice cream consumption per capita in our country is quite low compared to many other countries. However, the increasing number of modern ice cream-producing businesses, the rise in quality, and various educational activities have led to a growing trend in the consumption of this product during both summer and winter.

It is possible to make ice cream functional using various components. When considering the most commonly used methods, the types of functional ice creams can be listed as follows [14]:

- Probiotic, prebiotic, and synbiotic ice cream
- Ice cream enriched with whey
- Ice cream with reduced fat and/or sugar content
- Ice cream with increased antioxidant capacity
- Ice cream enriched with dietary fibers
- Ice cream enriched with omega-3 fatty acids
- Ice cream enriched with minerals
- Other functionally enhanced ice creams

•

Products containing probiotic bacteria and prebiotic substances hold an important place among functional foods because they help regulate the gastrointestinal system in individuals. Probiotics are live microorganisms that, when consumed in certain amounts, have positive effects on the gastrointestinal system of the individual [6]. It is known that probiotics present in the intestinal microflora have protective effects against various diseases, improve the immune system, have anti-carcinogenic properties, and lower serum cholesterol levels [5].

The combination of probiotics and prebiotics in a product is called synbiotic. If a probiotic bacterium utilizes a prebiotic present in the environment, a “synbiotic effect” occurs, and when a product containing both is consumed, the functional properties of both probiotics and prebiotics are utilized [5, 15].

There are numerous studies examining functional ice creams that result from the use of probiotics, prebiotics, or both, and their properties. In a study conducted by Alamprese et al. [16], the probiotic bacterium *Lactobacillus johnsonii* La1 was added to ice cream samples with varying fat and sugar contents, and various properties of the product were examined. The results showed that the probiotic bacteria used in ice cream production could maintain their presence at high levels for up to 8 months of storage, demonstrating that probiotic ice cream production is possible using the method in the study. In another study [17] where probiotic ice cream was produced from goat’s milk, the sample containing *Bifidobacterium animalis* subsp. *lactis* BLC1, a probiotic strain, was compared with a control sample without the bacteria in terms of various properties. The physical, chemical, and sensory analysis results indicated that producing probiotic ice cream from goat’s milk is feasible. Furthermore, in samples with bifidobacteria, approximately 7 log CFU/g of live microorganisms were detected within the first 24 hours after production, meaning 84.3% of the bacteria remained viable. During 120 days of storage under refrigeration, the number of live bacteria did not fall below 6.5 log CFU/g. In all studies, various microorganisms and components with probiotic or prebiotic properties were added to ice cream samples, and the physical, chemical, and sensory properties of these functional ice creams were examined. The results from all related studies demonstrated that the samples maintained their probiotic, prebiotic, or synbiotic properties throughout long storage periods.

Whey is the yellowish-green liquid portion separated when the curd is cut during cheese production [18]. Containing lactose, serum proteins, fat, minerals, and water-soluble vitamins, whey is a highly nutritious dairy byproduct. Due to its significant content of serum proteins with high biological value, whey has numerous health effects. Important biological effects of whey include antibacterial, antiviral, immune-supporting, antioxidant, anti-carcinogenic, and hypocholesterolemic effects. Research has also shown that it supports growth, bone development, and weight control [19, 20].

Milk fat has important functions, such as reducing the risk of heart disease and cancer, enhancing the immune system, and positively affecting bone health and body structure. Ice cream generally contains between 10-14% fat. Although milk fat has many positive effects, it is thought that consuming a product with such a high-fat content might pose health risks, especially for individuals with heart disease, cholesterol issues, and diabetes. In recent years, food products with reduced fat content have become more popular among individuals who need to consume less milk fat due to health problems and those who prioritize healthy eating. The sugar content of ice cream is also quite high, ranging between 15-17%. Consuming products with high sugar content is not suitable for individuals with conditions requiring reduced sugar intake, such as diabetes. People who care about their daily diet and want to lose weight also prefer food products with reduced sugar content.

Aime et al. [36] produced vanilla ice cream with reduced fat content using fat replacers to compensate for the textural defects caused by the reduction of milk fat. For this purpose, 5% pea starch was added as a fat replacer to each ice cream sample with varying fat levels (5%, 2.5%, and 0.4%) [21].

Antioxidants have protective and therapeutic effects on cells. Studies have shown that antioxidants exert a protective effect on cells by neutralizing free radicals [22]. Antioxidant compounds play a significant role in preventing many diseases, including cataracts, cancer, cardiovascular disorders, and neurological diseases. The most important natural antioxidant compounds include carotenoids, flavonoids, and other simple phenolic compounds found in cereals, fruits, and vegetables, along with vitamins A, C, and E [23]. Therefore, foods intended to be made functional with antioxidant properties often include various cereals, fruits, and vegetables high in these antioxidant compounds. There are several studies on the production of functional ice cream with increased antioxidant capacity and the investigation of its properties. For example, Hwang et al. [24] examined the effects of grape pomace on the rheological properties and antioxidant capacity of ice cream. As the amount of grape pomace added increased, the viscosity of the samples also increased, even surpassing that of samples with added stabilizers. Additionally, the melting rates of the samples significantly decreased with grape pomace addition. Another significant finding of the study was that the antioxidant capacity of ice cream samples increased due to the antioxidant content of grape pomace, and the antioxidant components remained stable during production.

Dietary fibers are food components primarily found in cereals, fruits, and vegetables that are highly resistant to digestive enzymes and are not digested in the small intestine but are partially or completely fermented in the large intestine. Fibers generally refer to the indigestible and relatively rigid parts of fruits and vegetables, such as peels, membranes, stems, and seeds. Dietary fibers, which have become increasingly prominent in low-energy diet products in recent years, are frequently used in functional foods due to their many positive health effects. It has been noted that dietary fiber components regulate large intestinal functions, have physiological effects on glucose and lipid metabolism and mineral absorption, and are known to have protective effects against constipation, hemorrhoids, colon cancer, obesity, diabetes, and cardiovascular diseases [25]. In a study conducted by Crizel et al. [26], some by-products obtained from oranges (peel, pomace, and seed fibers) rich in dietary fibers were used in ice cream, and various sensory properties of the samples were examined.

Functional foods enriched with products or components rich in omega-3 fatty acids can be placed at the forefront of foods that help reduce cardiovascular disorders. The most effective omega-3 fatty acids against these conditions are EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid), which are found exclusively in seafood and in trace amounts in other foods [27]. Besides cardiovascular benefits, EPA and DHA have numerous positive health effects. These fatty acids have been shown to prevent vascular blockage, gangrene, stroke, migraine, and allergic asthma, strengthen the immune system, and have protective and even therapeutic effects against various intestinal and skin diseases [28-29]. Due to these functional properties, the number of studies on products enriched with fish oil, especially, has been increasing. Additionally, the use of plant-based products rich in ALA (α-linolenic acid), another omega-3 fatty acid, such as flaxseed, purslane, spinach, soy, and canola, in foods is also possible. For the production of omega-3 enriched ice cream, Corradini et al. [30] used milk from cows fed with palm oil and coconut oil. Chromatographic analysis results showed that the milk from animals fed with palm oil contained more omega-3 fatty acids than milk from those fed with coconut oil, and the omega-3 fatty acid content of the ice cream samples mirrored the milk composition.

As previously mentioned, ice cream is a significant source of calcium and phosphorus [31]. However, as with almost all food products, the mineral enrichment of ice cream is a subject of study due to the important functions minerals serve. Minerals play general roles such as forming the basis of bone and tooth structure, being part of organic compounds like proteins and fats in muscles, organs, blood cells, and other soft tissues, and being involved in some enzyme systems, muscle and nerve stimulation, osmotic pressure, pH regulation, and acting as antioxidants. Various studies have been conducted on the mineral enrichment of ice cream due to these functional properties. In a study by Costa et al. [33], the effects of adding κ-carrageenan and calcium chloride on the structure and quality characteristics of ice cream were examined. The analysis results showed that calcium chloride addition caused growth in ice crystal size and partial coalescence in milk fat. Moreover, these effects were observed to be further enhanced with the addition of κ-carrageenan.

In addition to the various functional ice cream production methods mentioned above, research has also been conducted on ice cream enhanced with some natural flavoring agents, enriched with various fatty acids, and produced with reduced lactose content. For example, Trzecieski [34] conducted a study focused on reducing the lactose content of ice cream, which is often difficult for individuals with lactose intolerance to consume due to its high lactose content. In this study, lactase (β-galactosidase) enzyme preparation was added to the ice cream samples, facilitating the hydrolysis of lactose into glucose and galactose.

CONCLUSION

With changing living conditions, consumers' expectations from the foods they consume have evolved along with their eating habits. The desire for healthier eating, coupled with the growing demand to prevent diseases through diet, has increased significantly. Enhancing the functional properties of foods commonly consumed in daily diets, especially over the past decade, has become a frequently studied topic. The focus of these studies varies by country, with the type of food being functionalized or the aspect of functionality targeted based on the demands of individuals and the most common health issues in each region.

Prebiotics and probiotics are crucial for human digestive health, and many people prefer functional foods containing these beneficial bacteria because they need to include them in their daily intake. Antioxidants play an important role in metabolic processes and reactions. They act as hydrogen atom donors and convert chain-forming radicals into less reactive species, thereby preventing lipid peroxidation, protein cross-linking, and DNA mutations.

Ice cream has become a key focus for functional enhancement due to its nutritional value and its frequent inclusion in the diets of individuals of all ages. When examining the functional food groups consumed worldwide, functional dairy products are the most widely consumed in many countries, with ice cream holding a significant place within this category. Besides its high nutritional value, ice cream is a product enjoyed by people of all ages and, compared to other dairy products, is one of the easiest to modify in composition. This makes ice cream a popular subject of research for functional enhancement.

Educational activities for both producers and consumers regarding functional ice cream can further increase its consumption and lead to more focused research on this topic.

REFERENCES

[1] Açıkgöz, Z., Önenç, S., 2006. Fonksiyonel Yumurta Üretimi. Hayvansal Üretim 47(1): 36-46.

[2] Dayısoylu, K. S., Gezginç, Y., & Cingöz, A. (2014). Fonksiyonel gıda mı, fonksiyonel bileşen mi? Gıdalarda fonksiyonellik. *Gıda*, *39*(1), 57-62.

[3] Erbaş, M. (2006). Yeni bir gıda grubu olarak fonksiyonel gıdalar. Türkiye, 9, 24-26.

[4] Yaman, Ü. R., Kavas, N., & Yücel, U. (2012). Fonksiyonel Bir İçecek Olarak Şarap. Akademik Gıda, 10(3), 79-83.

[5] SAĞDIÇ, O., KÜÇÜKÖNER, E., & ÖZÇELİK, S. (2004). Probiyotik ve prebiyotiklerin fonksiyonel özellikleri. Research in Agricultural Sciences, 35(3-4).

[6] İnanç, N., Şahin, H., & Çiçek, B. (2005). Probiyotik Ve Prebiyotiklerin Sağlik Üzerine Etkileri. Erciyes Tıp Dergisi, 27(3), 122-127.

[7] Sezen, A. G. (2013). Prebiyotik, probiyotik ve sinbiyotiklerin insan ve hayvan sağlığı üzerine etkileri. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 8(3), 248-258.

[8] Özer, M., Özyurt, G., & Harsa, Ş. T. (2019). Probiyotik ve prebiyotiklerin bağırsak-beyin aksına etkisi. Akademik Gıda, 17(2), 269-280.

[9] Özyurt, V. H., & Ötleş, S. (2014). Prebiyotikler: metabolizma için önemli bir gıda bileşeni. Akademik Gıda, 12(1), 115-123.

[10] Karataş, N., & Şengül, M. (2018). Dut pekmezinin bazı kimyasal ve fiziksel özellikleri ile antioksidan aktivitesi üzerine depolamanın etkisi. Türk Tarım ve Doğa Bilimleri Dergisi, 5(1), 34-43.

[11] Meral, R., Doğan, İ. S., & Kanberoğlu, G. S. (2012). Fonksiyonel gıda bileşeni olarak antioksidanlar. Journal of the Institute of Science and Technology, 2(2), 45-50.

[12] SON, N. E. FONKSİYONEL GIDALAR. BESLENME OBEZİTE VE, 114.

[13] Gürsoy, A., 2007. Dondurma teknolojisi. In: Süt Teknolojisi, Edited by Yetişemiyen, A., Ankara Üniversitesi Yayınları No: 249, Ankara.

[14]Türkmen, N., & Gürsoy, A. (2017). Fonksiyonel Dondurma. Akademik Gıda, 15,(4), 388-392

[15] Holzaphel, W.H., Schillinger, U., 2002. Introduction to pre- and probiotics. Food Research International 35: 109-116.

[16] Alamprese, C., Foschino, R., Rossi, M., Pompei, C., Savani, L., 2002. Survival of Lactobacillus johnsonii La1 and influence of its addition in retailmanufactured ice cream produced with different sugar and fat concent

[17] da Silva, P.D.L., Bezerra, M.F., dos Santos, K.M.O., Correia, R.T.P., 2015. Potentially probiotic ice cream from goat's milk: Characterization and cell viability during processing, storage and simulated gastrointestinal conditions

[18] Koçak, C., 2007. Peynir teknolojisi. In: Süt Teknolojisi, Edited by Yetişemiyen, A., Ankara Üniversitesi Yayınları No: 249, Ankara.

[19] Karagözlü, C., Bayarer, M., 2004. Peyniraltı suyu proteinlerinin fonksiyonel özellikleri ve sağlık üzerine etkileri. Ege Üniversitesi Ziraat Fakültesi Dergisi 41(2): 197-207.

[20] Gür, F., Güzel, M., Öncül, N., Yıldırım, Z., Yıldırım, M., 2010. Süt serum proteinleri ve türevlerinin biyolojik ve fizyolojik aktiviteleri. Akademik Gıda 8(1): 23-31.
[21] Aime, D.B., Arntfield, S.D., Malcolmson, L.J., Ryland, D., 2001. Textural analysis of fat reduced vanilla ice cream products. Food Research International 34: 237 246. Aime, D.B., Arntfield, S.D., Malcolmson, L.J., Ryland, D., 2001. Textural analysis of fat reduced vanilla ice cream products. Food Research International 34: 237 246.
[22] Gökpınar, Ş., Koray, T., Akçiçek, E., Göksan, T., Durmaz, Y., 2006. Algal Antioksidanlar. E.Ü. Su Ürünleri Dergisi 23(1/1): 85-89
[23] Duell, P.B., 1996. Prevention of atherosclerosis with dietary antioxidants: fact or fiction?. The Journal of Nutrition 126: 1067-1071.
[24] Hwang, J.Y., Shyu, Y.S., Hsu, C.K., 2009. Grape wine lees improves the rheological and adds antioxidant properties to ice cream. Food Science and Technology 42: 312-318.
[25] Dülger, D., Şahan, Y., 2011. Diyet lifin özellikleri ve sağlık üzerindeki etkileri. U.Ü. Ziraat Fakültesi Dergisi 25(2): 147-157. [49] Soukoulis, C., Lebesi, D
[26] Crizel, T.M., Jablonski, A., Rios, A.O., Rech, R., Flôres, S.H., 2013. Dietary fiber from orange byproducts as a potential fat replacer. Food Science and Technology 53(1): 9-14.
[27] Uysal, K., 2007. Balık yağında bulunan ω-3 yağ asitlerinin farmakolojik özellikleri. Türk Sucul Yaşam Dergisi 5-8: 783.
[28] Eritsland, J., Arnesen, H., Seljeflot, I., Høstmark, A.T., 1995. Long-term metabolic effect of n-3 polyunsaturated fatty acids in patients with coronary artery disease. American Journal of Clinical Nutrition 61: 831-836
[29] Domingo, J.L., Bocio, A., Falcó, G., Llobet, J.M., 2007. Benefits and risks of fish consumption Part 1. A quantitative analysis of the intake of Omega-3 fatty acids and chemical contaminants. Toxicology 230(2-3): 219-226
[30] Corradini, S.A.S., Madrona, G.s., Visentainer, J.V., Bonafe, E.G., Carvalho, C.B., Roche, P.M., Prado, I.N., 2014. Sensorial and fatty acid profile of ice cream manufactured with milk of crossbred cows fed palm pil and coconut fat. Journal of Dairy Science 97(11): 6745-6753.
[31] Akın, N. 2009. Dondurma Bilimi ve Teknolojisi. Damla Ofset, Konya, Türkiye.
[32] Demirci, M., 1981. Sütün mineral maddeleri ve insan beslenmesindeki önemi. Atatürk Üniversitesi Ziraat Fakültesi Dergisi 12(1): 195-207
[33] Costa, F.F., Resende, J.V., Abreu, L.R., Goff, H.D., 2008. Effect of calcium chloride addition on ice cream structure and quality. Journal of Dairy Science 91(6): 2165-2174.
[34] Trzecieski, J., 1983. Lactose-reduced ice cream and process for the production thereof. US 4,374,861. Patent.

NEW TECHNOLOGIES AND TRENDS IN THE FOOD PRODUCT DEVELOPMENT PROCESS

Remziye Nur Gültekin, Orhan Onur AŞKIN

Kırklareli University

ABSTRACT

Today, manufacturing enterprises face numerous challenges, such as technological innovations, shortened product lifespans, increasing competition, and rapidly changing customer expectations. These challenges compel businesses to closely follow market developments and innovations, requiring them to adapt swiftly to maintain their competitive advantage. Especially with globalization, consumers have become more conscious and demand that food products are produced, processed, stored, transported, and distributed under safe conditions, emphasizing food safety. Customers prioritize not only the taste and quality of the products they purchase but also that these products are manufactured in a safe, healthy, and sustainable manner.

In this dynamic environment, businesses in the food industry are under pressure to prioritize food safety and nutritional quality while producing a diverse range of products that meet evolving customer needs. This pressure accelerates the new product development process in the industry and fosters the adoption of innovative approaches. In today’s food sector, the principle of sustainability is gaining increasing importance. In this context, environmentally friendly production methods, waste management, energy savings, and efficient use of natural resources are becoming key priorities for companies. New technologies and trends used in the food industry focus on extending shelf life while preserving the nutritional value of products, improving sensory characteristics such as taste and texture, and enhancing health-promoting functional components. These technologies include non-thermal food processing methods such as high-pressure processing, cold plasma technology, pulsed electric fields (PEF), ultrasonic and microwave processing, and other innovative techniques. Compared to traditional thermal processes, these methods better preserve the nutritional value of products and ensure food safety while causing less environmental harm.

Significant innovations are also observed in food packaging techniques. Solutions such as active and intelligent packaging, edible coatings, biodegradable packaging, and nanotechnology-enhanced packaging are being used to improve food quality and safety. These innovative packaging methods prevent food spoilage, reduce waste, and contribute to a more sustainable food system.

In conclusion, the new product development process in the food sector is shaped by innovative and advanced technological solutions that consider customer expectations and sustainability principles. These developments create a competitive advantage in the industry while supporting the community's access to healthy and safe food. Through these new technologies and sustainable practices, food manufacturers can respond more quickly to consumer demands, securing a strong position in the market.

Keywords: Food, new product, development, technology.

INTRODUCTION

The food sector holds a crucial position within the manufacturing industry due to the demand arising from one of the most fundamental human needs: nutrition. As it addresses the essential need for food products and is critically important for public health, the food sector is significant for both the economy and societal structure of a country. Additionally, the unique combination of both agricultural and industrial infrastructure within the food sector distinguishes it from other sectors.

The success of businesses in this field depends on enhancing the quality and reliability of the products they produce, expanding product variety, manufacturing in accordance with consumer orders, quickly meeting consumer needs and desires, and improving the services offered to customers. Achieving these objectives is possible only through the development of new products and their successful introduction to the market [1].

These are activities conducted to develop a new product or to produce an existing product more effectively or with improvements. For some, developing a new product means creating an entirely new product, while for others, it simply involves conducting scientific research. As the term "new product development" suggests, it involves initially conducting research to discover what has not yet been found and then enhancing or renewing the knowledge or product. It encompasses specialized activities aimed at making an existing product more effective or improved or developing a product that has never been produced but is planned for future production, with the goal of being a market leader in a particular field [2].

Innovative developments in food production systems contribute significantly to production efficiency. These innovations encompass a comprehensive process that begins with input procurement and extends through all production stages until the product reaches the consumer, even including after-sales activities. Both the public and private sectors have increasingly begun to invest efforts into the implementation of these innovative developments. The driving force behind this effort is the wave of innovation movements and globalization that have emerged worldwide in recent years. This ongoing transformation is evident in food production systems, and food production is increasingly becoming an integral part of industrial manufacturing [3].

The food sector is increasingly focusing on developing nutrient-rich, alternative, sustainable, and nanotechnological foods, as well as practices aimed at preventing waste, to keep pace with advancing technology. Among the developments aimed at meeting the growing demand for protein are alternative protein sources, such as lab-grown meat, insect-based proteins, and plant-based proteins. Following these developments, various plant-based protein products have emerged, including plant-based burgers, sausages, chicken, cheese, butter, eggs, and yogurt. Beyond these advancements, efforts are underway to meet the food needs of crews during space missions. Examples of such efforts include the development of long-shelf-life, nutrient-rich, and safe foods, often produced through freeze-drying methods [4].

1. Alternative Protein Sources

In recent years, the increasing human population and changing consumer preferences have led to a rise in research and biotechnological studies on alternative protein sources. It is estimated that with the growing human population, the demand for meat consumption will double, and animal production will reach its maximum capacity. Consequently, traditional methods are expected to fall short of meeting the rising demand, and production costs are anticipated to increase further. In this scenario, meat consumption is likely to be perceived as a luxury. Therefore, exploring new protein sources that can replace natural animal proteins, such as meat, is expected to have significant economic, nutritional, and environmental impacts and create substantial shifts in the meat industry [5].

The first commonly used alternative protein sources are plants and fungi [6]. Additionally, insects, seaweed, and cultured meat or in vitro meat also serve as significant protein sources [5].

1.1. Cultured Meat (In Vitro)

In vitro, or "cultured meat," also known as artificial or lab-grown meat, utilizes revolutionary technologies designed to address the challenges faced by the traditional meat industry [7]. Terms such as cultured meat, artificial meat, or clean meat are used for in vitro meat, which is derived from tissues and cells grown in a laboratory environment [5]. The development of artificial meat primarily focuses on two areas: cultured meat and plant-based meat alternatives. Several meat substitutes have been developed and are continuously being improved as alternatives to traditional meat [8]. For instance, meat substitutes made entirely from plant-based ingredients have gradually gained a small but growing market share [9]. Soy and wheat-based proteins are commonly selected for this production, providing efficient protein yields.

Although the technologies used to enhance the texture and taste of plant-based products are constantly evolving, replicating meat using plant-derived proteins, sugars, and fats remains challenging. Therefore, plant-based meat is predominantly used in processed meats such as burgers, sausages, and other minced products. Insects, on the other hand, are another natural protein source with high protein content in addition to sufficient minerals [8]. Insects contain between 38% and 77% protein by total dry weight. Many insect species, such as crickets (68.7%), have been reported to contain higher protein content per 100 g of dry weight than red meat (27.4%) or cod (28.5%) [10].

The potential benefits of cultured meat production include reducing greenhouse gases from animal production (especially methane), contributing to the prevention of global warming, preventing deforestation and land degradation, using agricultural land and crops to meet human nutritional needs, eliminating the necessity of slaughtering many animals, and meeting the protein needs of the growing human population [5]. Despite similarities to traditional red meat production, the acceptance of cultured meat by consumers remains controversial, as animals with artificially altered genomes in laboratories are considered artificial or human-made [7].

2. Nanotechnological Foods

The term nanotechnology is derived from the Greek prefix ‘nano,’ meaning ‘dwarf’ [11]. Food molecules found in nature exist on a micro or nanoscale. For instance, globular proteins in foods measure between 10-100 nm, and the 3-D crystalline structure of starch polysaccharides is 10 nm thick. In the food industry, many studies aim to create micro/nanoscale foods using top-down or bottom-up approaches. Nanotechnology is a relatively new field for the food industry. Nanotechnological applications in the food sector include nanoparticle delivery systems (such as micelles, liposomes, nanoemulsions, and biopolymeric nanoparticles), food safety, biosecurity (e.g., nanosensors), and nanotoxicology [12].

Nanofood is defined as foods derived through nanotechnological techniques or devices during soil processing, production, manufacturing, and packaging stages. Applications of nanotechnology in the food industry include improving food packaging systems to ensure quality and safe food production, enhancing traceability of food using biosensors, and developing active and intelligent packaging systems that allow the detection of bacteria [13].

One of the most common problems faced in the industry is the loss of freshness and quality of food before it reaches the consumer. One of the primary issues contributing to this is oxidation. Oxygen causes discoloration and spoilage of fats in cheese and meat. Polyamide-6 and ethylene vinyl alcohol can be used for food packaging; polyamide is suitable for less sensitive foods, while ethylene vinyl alcohol is ideal for highly sensitive foods. Some polymer researchers have developed a new nanocomposite plastic enriched with large-diameter silicate nanoparticles, known as a “hybrid system.” These hybrid systems have reduced oxygen permeability by 40% compared to conventional packaging materials. Nanotechnology plays a significant role in designing intelligent biosensors that can be packaged with food. These sensors, equipped with color-changing indicators, can inform consumers about the freshness of the food [12].

However, the use of nanoparticles in the food industry raises concerns among consumers. Due to the unknown effects of nanoparticles on human health and the lack of established maximum exposure limits, consumers are cautious about nanotechnology applications. It is believed that these concerns regarding the use of nanomaterials during food processing and packaging can be addressed through increased research, new approaches, and national and international legal and scientific regulations [13].

3. Space Food

Astronauts can face various nutritional deficiencies during space missions, making a healthy and nutritious diet a crucial factor for a successful space journey. Nutrition provides essential nutrients that help the body adapt to adverse environmental conditions (homeostasis), and nutrition in space differs from that on Earth due to the unique and harsh environment. Therefore, astronauts often encounter various nutrient deficiencies, including those required for their diets. To prevent these issues, space foods must be developed with critical parameters in mind. For example, these foods should have a longer shelf life and be produced to remain stable in zero-gravity conditions.

During space travel, astronauts must consume sufficient food not only to meet their nutritional needs but also to minimize the adverse effects of spaceflight on the body. Nutrition plays a vital role in countering the harmful effects of space travel on the human body, including oxidative stress, exposure to radiation, and bone and muscle loss [14].

Space food products typically contain reduced fiber and fat content and increased carbohydrates. The protein content is similar to that of Earth-based foods, but due to the absence of UV light, vitamin D intake is lower [14]. Changes in the body's biological clock and reduced food intake can alter taste perception, meaning that foods that taste good on Earth may not seem appealing in space [15]. To extend the shelf life of food and beverages in space, packaging must withstand pressure, external atmospheric conditions, and temperature while maintaining oxygen levels [16].

Flexible and soft packaging, suitable for sterilization, is often made from metal foil and flexible plastic laminates. These are used for packaging thermostabilized and irradiated foods, such as soups and dairy products, providing a long shelf life of up to 3-5 years, and serving as a replacement for rigid metal cans [14].

4. Three-Dimensional (3D) Food Production

Three-dimensional (3D) printers are increasingly gaining attention from both producers and consumers due to the innovations and advantages they offer. The primary reasons for this interest include the lack of need for special tools and equipment during production, reduction of labor, the ability to influence product quality characteristics (such as color, shape, texture, and aroma), and the ease of adjusting the raw material content as needed [17]. Today, raw materials developed specifically for this technology, as well as technologies that can process these materials appropriately, are commercially available [18].

In 3D food printers, the desired characteristic of raw materials is their ability to flow smoothly from the printer cartridge to the printing platform while maintaining a solid structure once deposited. Materials that can form hydrogel structures, such as starch and protein, as well as substances like cheese, chocolate, and hummus, can easily flow from the printer cartridge to the platform. In another study, sugar, starch, and mashed potatoes were used as raw materials, yielding positive results. It is worth noting that foods produced with these raw materials are generally in the snack or dessert category and are not suitable as main courses [17].

With materials that can be naturally printed in 3D printers, complete control can be achieved over taste, nutritional value, and texture. Although certain foods like meat, rice, fruits, and vegetables are inherently challenging to print, this limitation is being addressed. Aleph Farms, for instance, has developed a 3D bioprinting technology that uses animal cells to produce the world's first printed steak. To achieve a similar taste, tissues were taken from the steak portion of a cow and transformed into animal cells using incubators in a laboratory environment. Four different animal cells were obtained and prepared for use in the bio 3D printer. Regarding their produced steaks, the company stated, "The steak has a muscle structure and fat content similar to those of slaughtered cows and is as tender and delicious as meat you would buy from markets" [19].

These studies indicate the potential of 3D food printers. Today, consumer demand for boutique production and personalized food designs has reached significant levels. The industry's response to these demands, combined with expectations for precise and error-free production, the need to minimize raw material losses, and the requirement for skilled personnel, will form the basis for future developments in 3D food printers.

CONCLUSION

In the rapidly evolving food industry, innovations such as diverse food products, genetically modified foods, artificial meat and dairy products, edible insects, nanotechnology, 3D printers, and space food are contributing to meeting consumer needs and advancing existing technologies. These technological advancements not only cater to consumer demands but also offer significant benefits, such as improving product quality, reducing food waste, and providing alternative sources to address potential food access challenges arising from globalization.

It is anticipated that ongoing research and technological advancements in the coming years will further enhance these innovations, with consumers increasingly accepting and integrating the new developments brought about by these technologies into their daily lives.

REFERENCES

[1] Öztürk, D., & Onurlubaş, E. (2018). GIDA SEKTÖRÜNDE YENİ ÜRÜN GELİŞTİRME:
KONYA’DA BİSKÜVİ, ÇİKOLATALI VE ŞEKERLİ MAMULLER ALT SEKTÖRÜ ÜZERİNE BİR UYGULAMA. *Uluslararası İktisadi ve İdari İncelemeler Dergisi*, 551-568.
[2] ŞAHİN, A., & ARABACI, O. (2017). YENİ URUN GELİŞTİRME TAKIMLARINDA ORGUTSEL
ORTAMIN PROJE BAŞARISI VE PROJE HIZI UZERİNE ETKİLERİ. *Journal of International Social Research*, *10*(52).
[3] Adanacıoğlu, H., Saner, G., & Ağır, H. B. (2018). Sürdürülebilir gıda değer zinciri yaklaşımı. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *21*, 221-226.
[4] Aydın, M., Danacıoğlu, D. A., & Türker, S. (2021). GIDA TEKNOLOJİSİNDE YENİLİKÇİ
YAKLAŞIMLAR. *Helal ve Etik Araştırmalar Dergisi*, *3*(1), 19-36.
[5] Sürek, E., & Uzun, P. (2020). Geleceğin alternatif protein kaynağı: Yapay et. *Akademik Gıda*, *18*(2), 209-216.
[6] Van der Spiegel, M., Noordam, M.Y., Van der Fels-Klerx, H.J. (2013). Safety of novel protein sources (insects, microalgae, seaweed, duckweed, and rapeseed) and legislative aspects for their application in food and feed production.
[7] *Bonny, S. P. F., Gardner, G. E., Pethrick, D. W. & Hocquette, J-F. (2015). What is Artificial Meat and What Does It Mean for the Future of the Meat Industry? Journal of Integrative Agriculture, 14(2), 255-263.*
[8] Farhoomand, D., Okay, A., Aras, S., & Büyük, İ. (2022). Yapay et üretimi ve gelecek vizyonu. *Food and Health*, *8*(3), 260-272.

[9] Egbert, R., Borders, C. (2006). Achieving success with meat analogs. Food Technology (Chicago), 60(1), 28-34.
[10] Gahukar, R.T. (2011). Entomophagy and human food security. International Journal of Tropical Insect Science, 31: 129–144. doi10.1017/S174275841100025.
[11] Joseph, T. and Morrison, M., 2006. Nanotechnology in agriculture and food. Nanoforum report, Institute of Nanotechnology.
[12] Demirbilek, M. E. (2015). Tarimda ve gidada nanoteknoloji. *Gıda Ve Yem Bilimi Teknolojisi Dergisi*, (15).
[13] Saka, E., & Gülel, G. T. (2015). Gıda endüstrisinde nanoteknoloji uygulamaları. *Etlik Veteriner Mikrobiyoloji Dergisi*, *26*(2), 52-57.
[14] Ay, M., & Özdemir, E. (2023). Geleceğin Yemek Hizmetleri: Uzayda Beslenme Sistemleri. *ART/icle: Sanat ve Tasarım Dergisi*, *3*(1), 56-76.
[15] BHATIA, S. (2018). Predicting risk perception: new insights from data science. Management Science, Articles in Advance, 65(8), 1–24. doi: 10.1287/mnsc.2018.3121.
[16] *KIM, H.W. & RHEE, M.S. (2020). Space food and bacterial infections: Realities of the risk and role of science. Trends in Food Science & Technology, 106, 275–287. doi: 10.1016/J. TIFS.2020.10.023.*
[17] Sun J, Peng Z, Zhou W, Fuh JYH, Hong GS, Chiu A. 2015. A Review on 3D Printing for Customized Food Fabrication. Procedia Manufacturing, 1, 308–319.
[18] Değerli, C., & El, S. N. (2017). Üç boyutlu (3D) yazıcı teknolojisi ile gıda üretimine genel bakış. *Turkish Journal of Agriculture-Food Science and Technology*, *5*(6), 593-599.
[19] Akbaba, A. İ., & Akbulut, E. (2021). 3 Boyutlu Yazıcılar ve Kullanım Alanları. *ETÜ Sentez iktisadi ve idari bilimler dergisi*, (3), 19-46.

A DYNAMICAL EVALUATION OF THE EFFECTS OF FOREST PRODUCTS ON ECOLOGICAL FOOTPRINT: A NETWORK PERSPECTIVE

Semanur SOYYİĞİT[1]

[1] *Kirklareli University, Faculty of Economics and Administrative Sciences, Department of Public Finance, Kirklareli, Türkiye*
Corresponding author e-mail: semanur.soyyigit@klu.edu.tr

ABSTRACT

Based on the increase of severe climate conditions in recent years, sustainability and sustainable development has become a very important topic of the global agenda. Within this scope, ecological footprint has started being used as a tool to evaluate the damage caused by economic and social activities of human beings. There are six areas that ecological footprint tracks such as cropland, grazing land, fishing grounds, built-up land, carbon demand on land, and forest area. The measurement on forest area, namely Forest Product Footprint (FPF), reveals the impact of consumption on worldwide forests. There are especially nine primary commodities such as palm oil, coffee, soybean, cocoa, timber, rubber, meat, paper, and cardboard that have high risk of deforestation.
Based on the importance of the case, this study aims to examine the evolution of global trade of these commodities from 2000 to 2023. Complex network tools will be used to examine the global trade networks. Complex network analysis enables us to reveal some topological properties of the network such as connectedness, transitivity, core-periphery structure, power-law degree distribution etc. Network tools also provide some centrality measurements in order to evaluate the systemic importance of countries in the network. An analysis covering the period from 2000 to 2023 will help to gain a dynamical perspective and evaluation. We also aim to evaluate these centrality measurements by comparing the position of countries in global agreements on environmental issues.

Keywords: Ecological footprint, Cropland footprint, Palm oil, Global trade network, Panel data analysis

INTRODUCTION

Sustainability has become so much popular especially with the increase of the felt effects of climate change recently. However, the main idea behind it goes back to 1987 when the Our Common Future Report was published by the UN. In this report, some reasons and hope to believe in the success of humanity are addressed such as falling infant mortality, increasing life expectancy, increasing literacy rate etc. However, there is also a strong emphasis on the environmental threats that put the lives of many species, including the human, into risk (UN, 1987). Thus, sustainable development, used first in this report, implies "to meet the needs of the present without compromising the ability of future generations to meet their own needs" (UN, 1987). In the current climate, it is estimated that only 16% of the Sustainable Development Goals (SDGs) could have been achieved. The remaining SDGs exhibit very limited progress or reversal. In the same report, the targets, that are off-track, are related to food systems, sustainable land use, biodiversity, strong institutions, and peace (Sachs et al., 2024). Following carbon footprint that has the 60% share, cropland footprint ranks second with the 19% share in ecological footprint (WWF, 2022). The literature on sustainable development includes many studies about the causes and effects of carbon footprint. However, the studies on cropland footprint is quite limited. Hence, the focus of the present study is cropland footprint in general and palm oil trade in specific.

Based on this motivation, we conducted a two-step analysis. First, we examined the palm oil trade network with network analysis tools for each year from 2000 to 2022. This methodology enables us to reveal the topological properties of the network with a dynamical approach, and also to obtain centrality scores for each country that represent their export impacts in the global trade of palm oil. Secondly, depending on these centrality scores, we selected the top 8 exporter countries. Then, we applied panel data analysis to test the relations between the export impact and cropland footprint of these countries. The present study makes contribution to the existing literature since it combines two methodologies.

Based on this motivation, the study is structured as follows: Some conceptual explanation is given in Section 2. The results obtained from the analyses are presented in Section 3. In Section 4, there is an evaluation and conclusion of the study.

MATERIAL AND METHOD

Ecological footprint concept was first used and quantified in the mid-1990s. based on the fact that everybody has an impact on the Earth as they consume the nature, Wackernagel et al. (1997) developed a method to calculate it. Ecological footprint focuses on two roles of productive areas: to provide resource supplies uninterruptedly, and to absorb the waste generated during this production using the technological opportunities. Thus, calculations are also based on a simple logic with two facts (Wackernagel et al., 1997): (i) monitoring most resources we consume and wastes we generate, (ii) conversion of these resources and waste flows into biologically productive areas to provide these functions properly. As land use contributes to produce different goods and services in a non-substitutable way, ecologically productive areas are divided into six categories such as arable land, forests, pasture, sea, built up area, and fossil energy land (Wackerhanel et al., 1997). Currently, we can reach the measurements of ecological footprint in total and also in these six categories, namely cropland footprint, grazing land footprint, forest products footprint, fishing grounds footprint, built-up land footprints and carbon footprint (Global Footprint Network, 2016). In the ecological footprint calculations, areas accepted 'unproductive for human purposes' (i.e. deserts and ice layer) are excluded (Lenzen and Murray, 2003). However, not all that biologically productive land area is available to human use since this area is also home to millions of spaces sharing this planet. Thus, it is stated that at least 12% of the ecological capacity should be preserved to protect the biodiversity (Wackernagel et al., 1997).

On the other hand, agricultural and forestry activities are accepted as the main causes of biodiversity loss. The demand for the products of these sectors increases as a result of increasing population and economic development (Marques et al., 2019). Therefore, the conversion of pasture to cropland area causes biodiversity loss. As a result of this loss, a planetary boundary is proposed for croplands as 15% of this productive land area (Ridoutt and Garcia, 2020).

The calculations indicate that carbon footprint has the highest impact on ecological footprint, and it is followed by cropland footprint (WWF, 2022). In cropland footprint, palm oil has a special importance as this sector contributes to high cropland loss among pulses and oil crops. Major producer countries exploit more land to fulfil their production targets (Malahayati, 2022).

Palm oil currently accounts for 41% of vegetable oil production around the world. As a result, it is criticised to be a major contributor to biodiversity loss and greenhouse gas emissions due to the conversion of lands (Beyer et al., 2020). It is documented that the global cultivation area for palm oil has increased almost 23% for the last five years (Patel, et al. 2022). Considering this increase of palm oil in the land area, more attention has been paid to the palm oil related economic activities such as, production, trade, consumption etc. Based on this, we analyse international trade of palm oil and its relation with cropland footprint.

For this purpose, this study has a two-step analysis. At the first step, network analysis has been applied to the international trade data of palm oil based on export from 2000 to 2022. This analysis enables us to examine the topological properties from a network perspective with a dynamical approach. This analysis also gives us centrality score (i.e. export impact score) for each country in the network. We selected top 8 countries with the highest centrality scores, and then built the panel data analysis on this scores. At the second step, we search for the cointegration between the export impact and cropland footprint.

RESULTS AND DISCUSSION

Network analysis results

Fig. 1. presents the trend of the density coefficient through the period. Density coefficient, which is the ratio of actual links among the nodes to the maximum possible number of the links (Newman, 2010), is low so that the international trade network of palm oil is a sparse network. However, an increasing trend is observed during the period.

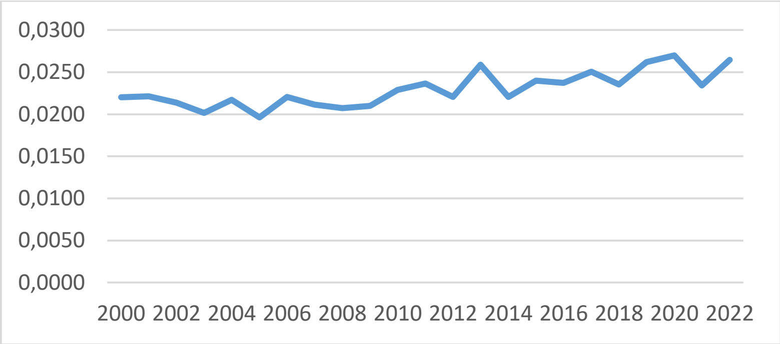


Fig. 1. Density coefficient

Source: Drawing is based on the author's calculation

Table 1 presents another important results from network analysis. Kolmogorov-Smirnov test is used to test whether the distribution of links follows a power-law distribution. If p-value is lower than 0.05, we reject the null hypothesis that it follows power-law (Igraph, 2020).

Table 1. Kolmogorov-Smirnov test results

Years	Skewness	Kurtosis	α	KS stats	KS p-value
2000	9.621	97.270	2.073	0.132	0.999
2001	7.288	56.910	1.371	0.125	0.773
2002	8.702	81.503	1.341	0.110	0.818
2003	8.533	78.676	1.235	0.124	0.441
2004	8.861	84.483	1.298	0.091	0.895
2005	9.603	98.318	1.263	0.095	0.834
2006	8.749	82.024	1.331	0.097	0.888
2007	9.905	104.386	1.230	0.107	0.605
2008	10.282	111.615	1.351	0.118	0.832
2009	10.440	114.826	1.395	0.138	0.705
2010	10.282	111.462	1.272	0.112	0.700
2011	9.543	97.626	1.170	0.138	0.218
2012	8.637	78.408	1.174	0.119	0.334
2013	8.277	72.867	1.749	0.121	0.995
2014	8.169	68.888	1.183	0.123	0.336
2015	8.260	71.439	1.208	0.111	0.505
2016	8.071	68.749	1.243	0.132	0.336
2017	9.633	100.223	1.253	0.121	0.416
2018	8.950	86.783	1.517	0.112	0.947
2019	8.807	83.891	1.210	0.128	0.383
2020	8.218	72.366	1.179	0.130	0.270
2021	8.728	84.004	1.190	0.128	0.237
2022	7.354	58.776	1.196	0.113	0.434

Source: Author's calculation.

It is observed that the international trade network of palm oil follows a power-law distribution for each year, so that the connectedness of the network is heterogeneous.

As an another important property, Fig. 2 includes results from core-periphery analysis. Following the methodology developed by Borgatti and Everett (1999), the values in Fig. 2 refer to the similarity of the network in question to an ideal core-periphery network. Accordingly, similarity to an ideal core-periphery network, meaning that there are some core countries strongly connected to one another and also peripheral countries while peripheral nodes are not connected themselves, has an increasing trend.

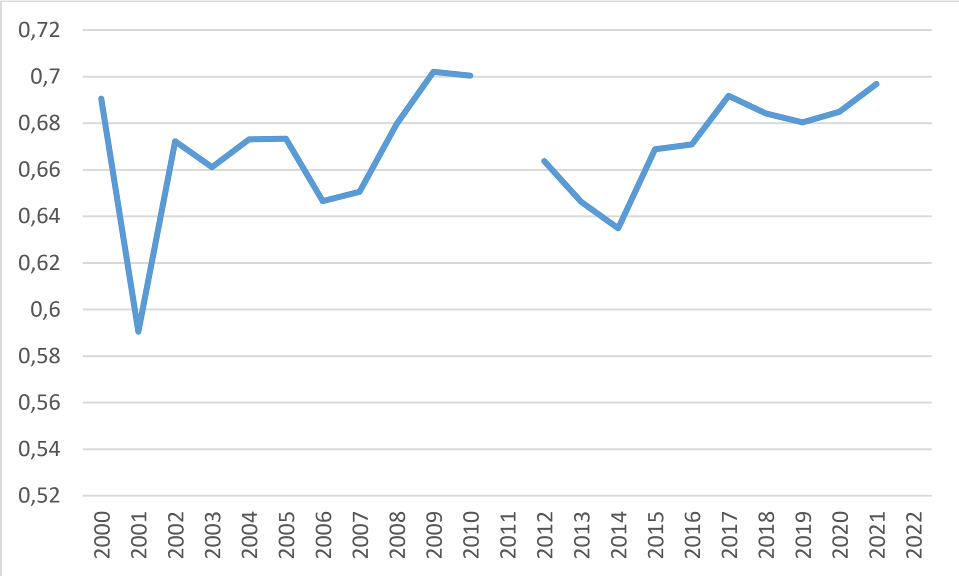
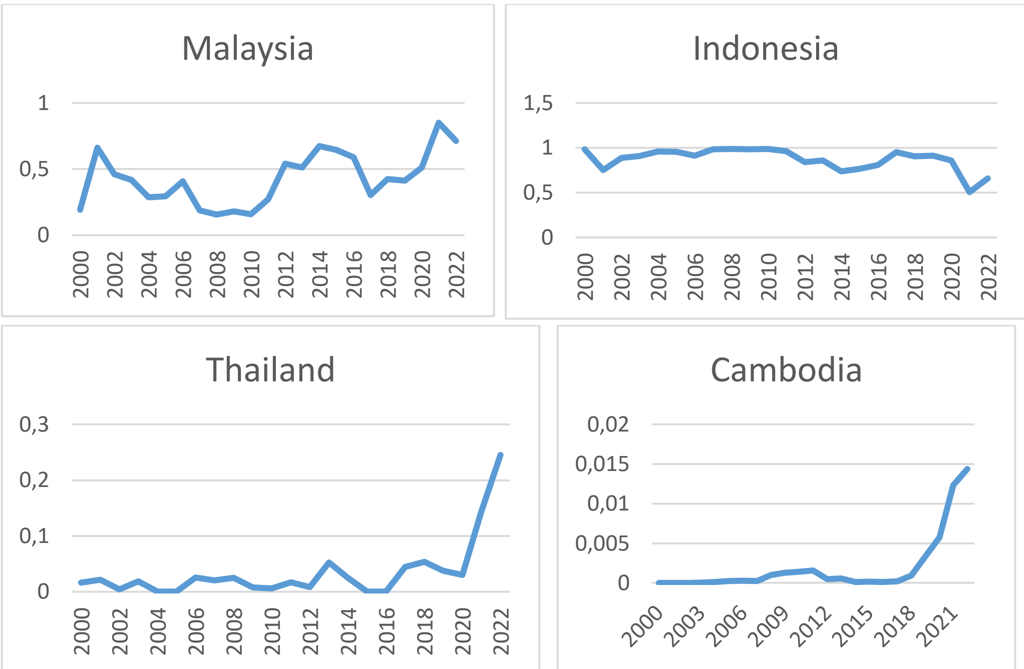


Fig. 2. Core-periphery fit correlation
Source: Drawing is based on the author's calculation

As a final important part of a network analysis, we present centrality scores for top exporter countries. among many centrality measurement, we applied hub centrality developed by Kleinberg (1999). Hub centrality corresponds to 'export impact' for this network. The graphs in Fig. 3 indicate the export impacts of the top eight exporter countries. it is observed that all countries have an increasing export impact within the network except for Indonesia.



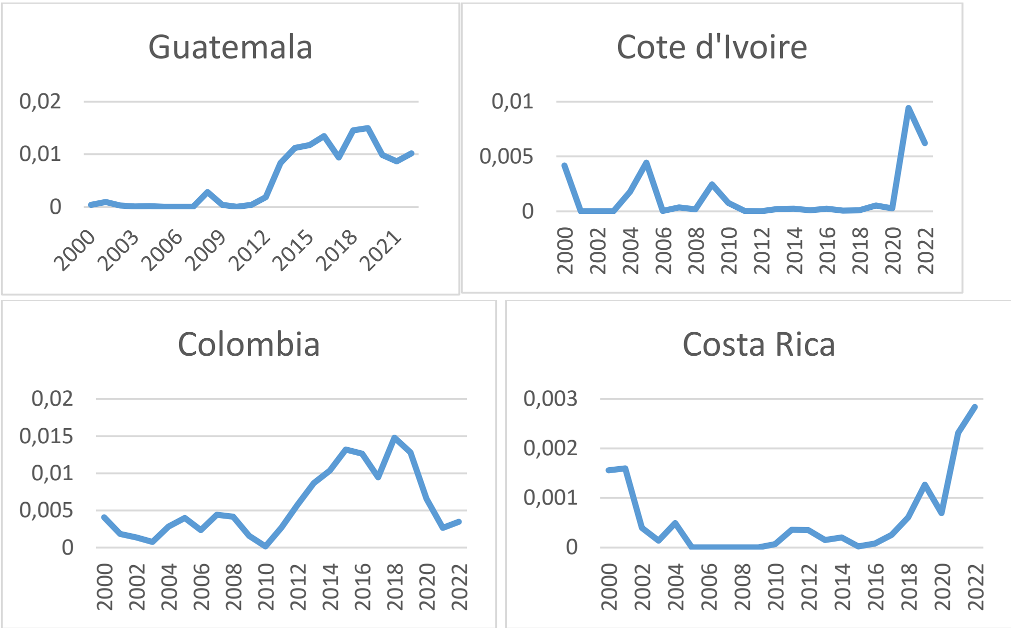


Fig. 3. Hub centralities based on export
Source: Drawing is based on the author's calculation

These findings give an idea about the network topology. However, these findings are also an input for the second step of the analysis. We searched for the long-run relationship between hub centralities (export impacts) of the countries and cropland footprint. The research question for the second part of the examination is whether export impact has an impact on cropland footprint in the long-run.

Panel Data results

The model we focus on in this part of the analysis is as follows:

$$\text{Crop=f (Hub)} \qquad (1)$$

However, we need to apply some preliminary tests before cointegration. First of all, we presented cross-section dependence test results for the variables. Accordingly, the null hypothesis of no cross-section dependence is rejected for each variable. Based on this results, we chose CADF test as a second generation unit-root test.

Table 2. Cross-section dependence test results for variables

Variables	CDLM 1	CDLM 2	CDLM adj
HUB	196.970	21.511	21.329
	(0.000)	(0.000)	(0.000)
CROP	165.638	17.324	17.142
	(0.000)	(0.000)	(0.000)

Unit-root test in Table 3 indicate that both variables have unit root at level, and become stationary at first difference. This result makes us consider whether there is a cointegration between these series.

Table 3. CADF panel unit-root test results

Variables	CIPS stats	
	Constant	Constant and trend
HUB	-1.281	-1.977
Δ HUB	-4.092***	-4.161***
CROP	-1.888	-3.180***
Δ CROP	-4.897***	-

Before testing cointegration, we applied a cross-section dependence test to the residuals of the model. The results are given in Appendix. Accordingly, we cannot reject the null hypothesis of no cross-section dependence. Based on this result, we applied first-generation version of Westerlund (2007) cointegration test. The results in Table 4 verify that there is cointegration between the variables in the model.

Table 4. Westerlund (2007) cointegration test results

Model	Statistics	Value	P-value
CROP = F (HUB)	Gt	-3.033***	(0.000)
	Ga	-10.489**	(0.043)
	Pt	-6.01**	(0.035)
	Pa	-6.825*	(0.064)

Before estimation of the coefficient, we first tested the homogeneity of the slope coefficients of the panel. The results obtain from the homogeneity test are given in Appendix. Accordingly, we reject the null hypothesis of homogeneity, meaning that coefficients are heterogeneous in the panel.

Table 5. Long-run coefficient estimates

Countries	DOLS			FMOLS			CCR		
	Beta (Variable: HUB)	Std. Error	t-stat	Beta (Variable: HUB)	Std. Error	t-stat	Beta (Variable: HUB)	Std. Error	t-stat
Malaysia	**1.69**	**0.26**	**6.4**	**1.57**	**0.2**	**7.71**	**1.56**	**0.2**	**7.89**
Indonesia	**0.48**	**0.02**	**25.38**	**0.5**	**0.03**	**16.28**	**0.5**	**0.03**	**16.29**
Thailand	**12.94**	**3.69**	**3.51**	**7.37**	**2.18**	**3.38**	**6.7**	**1.97**	**3.39**
Cambodia	375.5	251.82	1.49	**124.98**	**52.72**	**2.37**	**122.09**	**51.66**	**2.36**
Guatemala	33.94	23.1	1.47	39.8	20.33	1.96	39.4	20.24	1.96
Cot'e d'Ivoire	**231.8**	**111.08**	**2.09**	125.81	67.51	1.86	110.76	60.26	1.84
Colombia	**54.26**	**6.94**	**7.82**	**57.7**	**9.61**	**6**	**56.45**	**8.93**	**6.32**
Costa Rica	148.93	708.71	1.37	**134.1**	**574.19**	**2.35**	**115.1**	**48.68**	**2.37**
Model	274.07	17.51		213.35	14.82		186.11	15	

Then, long-run coefficients have been estimated by using FMOLS, DOLS and CCR methods that are first generation estimators. According to the results from DOLS estimator, export impact has a positive effect on cropland footprint in Malaysia, Indonesia, Thailand, Cot'e d'Ivoire and Colombia at 0,05 significance level. FMOLS and CCR results indicate that export impact has a positive effect on cropland footprint in Malaysia, Indonesia, Thailand, Cambodia, Colombia and Costa Rica at 0,05 significance level.

CONCLUSIONS

Cropland footprint has the second highest impact on ecological footprint following carbon footprint. In cropland footprint, palm oil has a special importance as it causes high cropland loss among all oil crops. Top exporters of this crop are claimed to convert more and more land to produce more palm oil. Hence, we aim to examine whether export centralities of top exporters affect their cropland footprint.

For his purpose, we first applied network analysis. Results obtained from network analysis indicate an increasing trend of connectedness and a trade structure clustered around some super hubs. This analysis also provides us centrality score for all countries that refers to export impacts of countries. Afterwards, we applied panel data analysis to examine whether a relation between export impact and cropland footprint in the long-run. The results approve the existence of such a long-run relationship between these variables. Accordingly, the effect of export centrality on cropland footprint is positive for top

exporters of this product. In the top three exporters, namely Malaysia, Indonesia and Thailand, an increase in the export impact also increases cropland footprint.
This result approves the argument mentioned in the literature. The conversion of other areas to cropland areas results in biodiversity loss and increasing greenhouse gas emissions, hence increase in cropland footprint. Considering the planetary boundary that is proposed for croplands as 15% of this productive land area, policy implementations are required to be taken urgently in order to prevent more biodiversity loss and cropland footprint.

REFERENCES

Beyer, Robert M., America P. Duran, Tim T. Rademacher, Philip Martin, Catherine Tayleur, Sharon E. Brooks, David Coomes, Paul F. Donald and Fiona J. Sanderson. 2020. The environmental impacts of palm oil and its alternatives. BioRxiv. https://doi.org/10.1101/2020.02.16.951301

Borgatti, S.P.& Everett, M.G. 1999. Models of core/periphery structures. Social Networks, 21, 375-395.

Global Footprint Network. 2016. Living Planet Report 2016 – Technical Supplement: Ecological Footprint.

Igraph (2020). http://igraph.org/r/doc/fit_power_law.html . (accessed 31 August 2024).

Kleinberg, J.M. 1999. Authoritative Sources in a Hyperlinked Enironment. auth.pdf (cornell.edu). (accessed 31 August 2024).

Lenzen, Manfred and Shauna A. Murray. 2003. The ecological footprint – Issues and trends. ISA Reseach Paper 01-03.

Marques, Alexandra, Inês S. Martins, Thomas Kastner, Christoph Plutzar, Michaela C. Theurl, Nina Eisenmenger, Mark A.J. Huijbregts, Richard Wood, Konstantin Stadler, Martin Bruckner, Joana Canelas, Jelle P. Hilbers, Arnold Tukker, Karlheinz Erb, and Henrique M. Pereira. 2019. Increasing impacts of land-use on biodiversity and carbon-sequestration driven by population and economic growth. Nat Ecol Evol. 3(4): 628-637, doi:10.1038/s41559-019-0824-3.

Newman, M.E.J. 2010. Networks An Introduction. Oxford University Press.

Patel, Seeta Salgia, Aviva Intveld, Evan Seeyave, Emily Moberg and Virginia Barreiro. 2022. Measuring and Mitigating GHGs: Palm Oil. WWF.

Sachs, Jeffrey D., Guillaume Lafortune and Grayson Fuller. 2024. Sustinable Develoment Report 2024 – The SDGs and the UN Summit of the Future: Includes the SDG Index and Dashboards. Dublin University Press, Ireland.

Schaefer, Florian, Ute Luksch, Nancy Steinbach, Julio Cabeça and Jörg Hanauer. 2006. Ecological Footprint and Biocapacity – The world’s ability to regenaret resources and absorb waste in a limited time period. European Commission Working Paper.

UN. 1987. Report of the world commission on environment and development: our common future.

Wackernagel, Mathis, Larry Onisto, Alejandro Callejas Linares, Ina Susana Lopez Falfan, Jesus Mendez Garcia, Ana Isabel Suarez Guerrero, Ma. Guadalupe Suarez Guerrero. 1997. Ecological footprints of nations – How much nature do they use? How much nature do they have?.

WWF. 2022. Living planet report 2022 – building a nature-positive society. Eds. Almond, R.E.A., Grooten M., Juffe Bignoli, D. and Petersen, T. Switzerland: Gland.

Appendix

Model: CROP = F (HUB)			
Cross-section dependence test	CDLM 1	36.66	(0.126)
	LM adj	1.868	(0.062)
Homogeneity test	Delta	4.698***	(0.000)
	Delta_adj	5.039***	(0.000)

EFFECT of ASPROSIN HORMONE on SKMEL-30 CELLS

Nebiye Pelin TURKER

[1] *Trakya University, Technology Research Development Application and Research Center (TÜTAGEM), Edirne, Turkey*
npelinturker@trakya.edu.tr

ABSTRACT

SKMEL-30 melanoma cancer is among the deadliest kind. Paying attention to SKMEL-30's altered energy metabolism and enhanced aerobic glycolysis is necessary. Insulin resistance and metabolic diseases are frequently accompanied by dysregulation of the glucogenic hormone asprosin. Its involvement in energy metabolism in the tumor microenvironment has not yet been studied, despite its link to metabolic diseases. Methods: MTT analysis, spectrophotometric measurement of sialic acid levels, and qRT-PCR for determining glycosyltransferase expression. Results: The ST8SIA-2 gene was significantly upregulated in the SKMEL-30 cell line after treatment with 50 nM asprosin ($p < 0.05$). It has been demonstrated that asprosin affects glucose and energy metabolism pathways as well as those linked to cell division and communication. Asprosin was found to lower the sialic acid level when sialic acid levels were examined at the control and 50 nm asprosin dose. In conclusion: Asprosin seems to control the glycosyltransferases' in vitro signaling pathway with regard to SKMEL-30. The Warburg effect, or aerobic glycolysis, is a role in the development of numerous malignancies, including SKMEL-30. Even in the presence of oxygen, the glycolytic pathway—which uses glucose for quick energy production and generates excess lactate—is frequently chosen over aerobic respiration and oxidative phosphorylation when dealing with excess glucose. It's common knowledge that breathing on its own can keep tumors alive. Oncogenesis begins with aberrant growth factor signaling regulation, which is a modifiable element in aerobic glycolysis. As a glucogenic hormone, asprosin is believed to play a part in this process and is a good subject for further study in the tumor microenvironment.

Keywords: SKMEL-30, Melanoma, Glycosyltrasnferases, Sialic Acid, Apoptosis

INTRODUCTION

Nowadays, the idea that cancer is a hereditary illness is starting to be viewed more and more as a metabolic disorder. Regardless of its cellular or tissue origin, emerging data is consistent with the overall idea that cancer is a disease in which cellular energy metabolism is disturbed (Hainaut and Plymoth; 2012). Due to specific metabolic processes that have been reprogrammed, cancer cells have unique characteristics such as dysregulated lipid metabolism and fast uptake of glucose (Jones and Thompson; 2009). Generally speaking, the primary adaptation mechanism that encourages tumor cell proliferation and survival is the Warburg effect (Figure 1). In this instance, preferential lactate generation coupled with an enhanced rate of glucose absorption permits unchecked growth and preservation of the tumor microenvironment even in the presence of aerobic glycolysis (Makale; 2008).

Numerous adipokines may stimulate cell migration and proliferation as well as other anti-apoptotic pathways, according to research investigations, which may accelerate the development of tumors and the spread of cancer (Booth et al.; 2015). On the other hand, there aren't many data on asprosin peptide levels in cancer. Remarkably, Kerslake et al. found that asprosin and its conjugated olfactory receptor OR4M1 were highly expressed in both malignant ovarian tissues and normal human ovaries, and they also showed that this had an effect on the tumor microenvironment. A recent in vitro investigation conducted by the same group on the ovarian cancer cell line SKOV-3 revealed differential regulation of genes after treatment with 100 nM asprosin (Kerslake et al.; 2022). Furthermore, asprosin changed several signaling pathways linked to cell division and communication as well as phosphorylated ERK1/2 (Kerslake et al.; 2022). It has previously been reported that the Warburg effect may have an effect on the tumor microenvironment in ovarian cancer. Other functionally expressed genes, such as Cadherin 11 (CDH11) and the Fc fragment of immunoglobulin G receptor IIa (FCGR2A), have been implicated in the regulation of metastatic progression and chemotherapeutic response in different cancers. (Wang et., 2017; Qian et al., 2021).

Cachexia syndrome (CACS) is a term for the starvation that many cancer patients may experience as their disease worsens. Depending on the type and stage of their tumor, CACS prevalence rates can range from 9% to 85%. Arends and Van Cutsem, 2005). According to Fearon et al. (2011), it is characterized by energy imbalance, systemic inflammation, and involuntary loss of lean body mass with or without adipose tissue wasting. Adipose tissue is decreased in anorexia as a result of caloric deprivation, whereas in CACS, skeletal muscle and adipose tissue degenerate (Tisdale, 1997). When compared to non-anorexic persons, cancer patients with anorexia showed significantly lower levels of asprosin, but not CACS (Du et al., 2021). To fully understand the potential function of asprosin in the initiation and spread of cancer, more research is required.

Our research aimed to understand how asprosin hormone affected sialic acid changes and signaling pathways in SKMEL-30 melanoma cancer cells. The LD50 dose of asprosin hormone was established by performing the MTT assay on SKMEL-30 cells at various doses. Next, qRT-PCR research was carried out to look into the genes for glycosyltransferase, apoptotic, and metastatic processes in order to assess the alterations brought about by the LD50 dose in the cell. Additionally, sialic acid levels were assessed by comparing them to the control group in order to look at the potential for SKMEL-30 cells treated with asprosin hormone to spread.

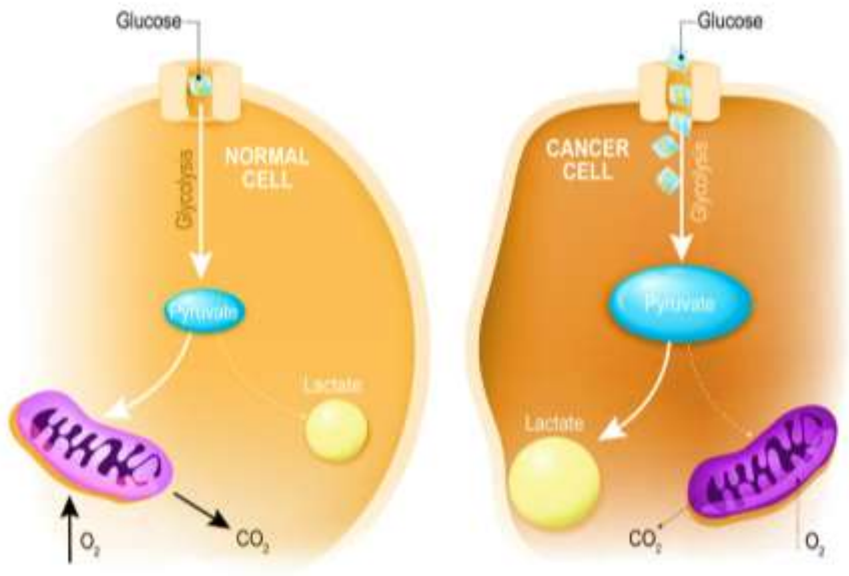


Figure 1. Warburg effect in healthy and cancerous cells

MATERIAL and METHODS

Cell Culture analysis

In this investigation, the melanoma (SKMEL-30) cell line was employed. The Safe Fast Elite (EN 12469 2000) flow chamber was used to cultivate the cell line. Bovine serum-containing media (MULTICELL (FBS-HI-IIA)) was used for cell passage and culture, together with DMEM, EMEM-HamsF-12 (MULTICELL), 1% L-glutamine (MULTICELL (609-065-E2), and 1% penicillin-streptomycin (MULTICELL (450-201-Z2)). The medium was kept at 37 °C and 5% CO2 in sterile incubators (PANASONIC). Up until the fifth passage, cultured cell lines were repeated; at that point, stocks were prepared for utilization. DMSO (MERCK 67-68-15) was frozen in liquid nitrogen, added to medium-containing cryotubes, and maintained at -150°C (PANASONIC). Every study project started at the fifth cell line passage and finished at the fifteenth. Using the MTT method, cell viability and proliferation were assessed. 2000 SKMEL-30 cells per well were seeded on cell culture plates in a 96-well plate. It was incubated for 24 hours in DMEM:EMEM:Ham's F12 medium to support cell adhesion. The range of dosages for asprosin hormone administration was identified by searching the literature. Irisin hormone was diluted serially in ultrapure water within the range of 3.75-120 nanoMolar (nM). During the course of a 24-hour incubation period, eight replicates of each dose were tested in treated cells. After that, each well received 20 microliters of MTT solution (5 mg/ml concentration), and each well was incubated for two hours at 37°C.

Measuring the Levels of Sialic Acid

Following the planting of SKMEL-30 cells on 6-plate plates, the cells were cultured for 24 hours at 37°C with 5% CO2. At the end of the interval, 30 nM asprosin hormone was added and the mixture was incubated for 24 hours. For five minutes, control and SKMEL-30 cells treated with 50 nM asprosin were homogenized in 0.1 M phosphate buffer (pH 7.4). After 0.2 ml of homogenized samples were collected, 1 ml of a 5% perchloric acid solution was added, and the samples were incubated for 5 minutes at 100°C. Phospholipid buffer (0.1 M–pH 7.4) was used to make a 2.5 mg/ml sialic acid (N-acetyl neuraminic acid) solution. The prepared stock solution was diluted, and standard operating procedures were developed. The samples were incubated with Ehrlich's reagent for four hours at 100°C after it was added to the calibration procedures. Graphs were made and the concentrations of sialic acid in the samples were determined in milligrams per milliliter based on spectrophotometric measurements obtained at 525 nm.

RNA isolation, cDNA synthesis and Real-Time PCR (qRT-PCR) Analysis

Using the Pure Link RNA isolation kit, total RNA was extracted from the SKMEL-30 cell line (Invitrogen TM-12183018A). The amount of RNA that was isolated was measured using NanoDrop (NanoQ Optizen). Applied Biosystems 00709629 cDNA Reverse Transcription Kit PCR circumstances The steps for completing cDNA synthesis were as follows: Step 1 was 25°C for 10 minutes; Step 2 was 37°C for 120 minutes; and Step 3 was 85°C for 5 minutes. A Quantstudio 6 Flex qRT-PCR system (Sybr Green Method (PowerSYBR Green-Applied Biosystems-1805575)) that reads 384-well microplates was used in this experiment. GAPDH was the endogenous gene used to analyze the samples.

Statistical analysis

The formula "Cell Viability%: (absorbance value of the substance applied wells / absorbance value of the control wells) × 100" was used to determine the amount of cells due to irisin application. The tool SPSS Regression Probit (IBM SPSS Statistics 22) was used to compute the LD50 doses. Gene expression changes resulting from three consecutive LD50 dosages were computed using the formula $2^{-\Delta\Delta cT}$. The statistical difference between the results was calculated using the SPSS-Anova (Duncan Test) with a p-value of less than 0.05. mRNA for GAPDH was employed as an internal control.

RESULTS AND DISCUSSION

Based on the study results, A 50 nm asprosin dosage in MTT measurement resulted in 50% of the cells dying. The ST8SIA-2 gene, particularly one of the glycosyltransferase genes, significantly increased in the SKMEL-30 cell line after treatment with 50 nM asprosin ($p < 0.05$). The asprosin group showed a 10,478-fold rise whereas the control group showed a 2,557-fold increase. Graphs of the glycosyltransferase and endoplasmic reticulum stress genes studied are presented in Figure 2. It has been demonstrated that asprosin affects glucose and energy metabolism pathways as well as those linked to cell division and communication. Asprosin was found to lower the sialic acid level when sialic acid levels were examined at the control and 50 nm asprosin dose. Asprosin seems to control the glycosyltransferases' in vitro signaling pathway with regard to SKMEL-30. As part of the study's parameters, the MTT test—which is covered in the Materials and Methods section—was employed to evaluate the effect of asprosin hormone on the 24-hour viability of SKMEL-30 melanoma cell lines. Asprosin hormone exposure for 24 hours resulted in dose-related mortality on cell viability for the SKMEL-30 cell line. The administration of asprosin hormone produced a 50 nM LD50 dose. A significant decrease in silicic acid levels (mean±std.) was observed between SKMEL-30 cells treated with 50 nM asprosin hormone and the control group. (It was determined as 0.4173 mg/mL in the control and 0.2456 mg/mL in the asprosin group). These results; This suggests that asprosin stops cell proliferation by suppressing the metastatic ability of SKMEL-30 cells.

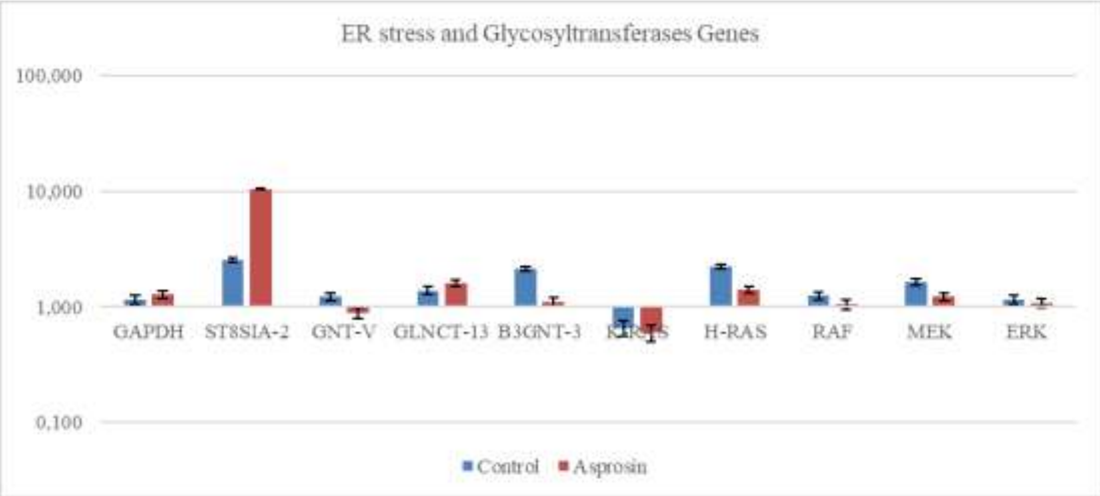


Figure 2. Results for glycosyltransferase and Endoplasmic reticulum stress genes.

Apoptosis genes: Cas-8 and Cas-9 showed a statistically significant increase ($p<0.05$). In the control and asprosin groups, the Cas-8 gene grew 11,165 and 84,755 times, respectively; in contrast, the Cas-9 gene increased 1,058 and 1,701 times, respectively. The flow cytometry data show that this rise is not, however, at a level that will cause the cells to undergo apoptosis. Metastasis genes: TIMP-1 and CDH-1 showed a statistically significant increase ($p<0.05$) in comparison to the control. While a rise of 0.695 and 1.622 times was seen in the CDH-1 gene, an increase of 0.816 and 1.279 folds was seen in the TIMP-1 gene. The results for apoptosis and metastasis genes are presented in Figure 3.

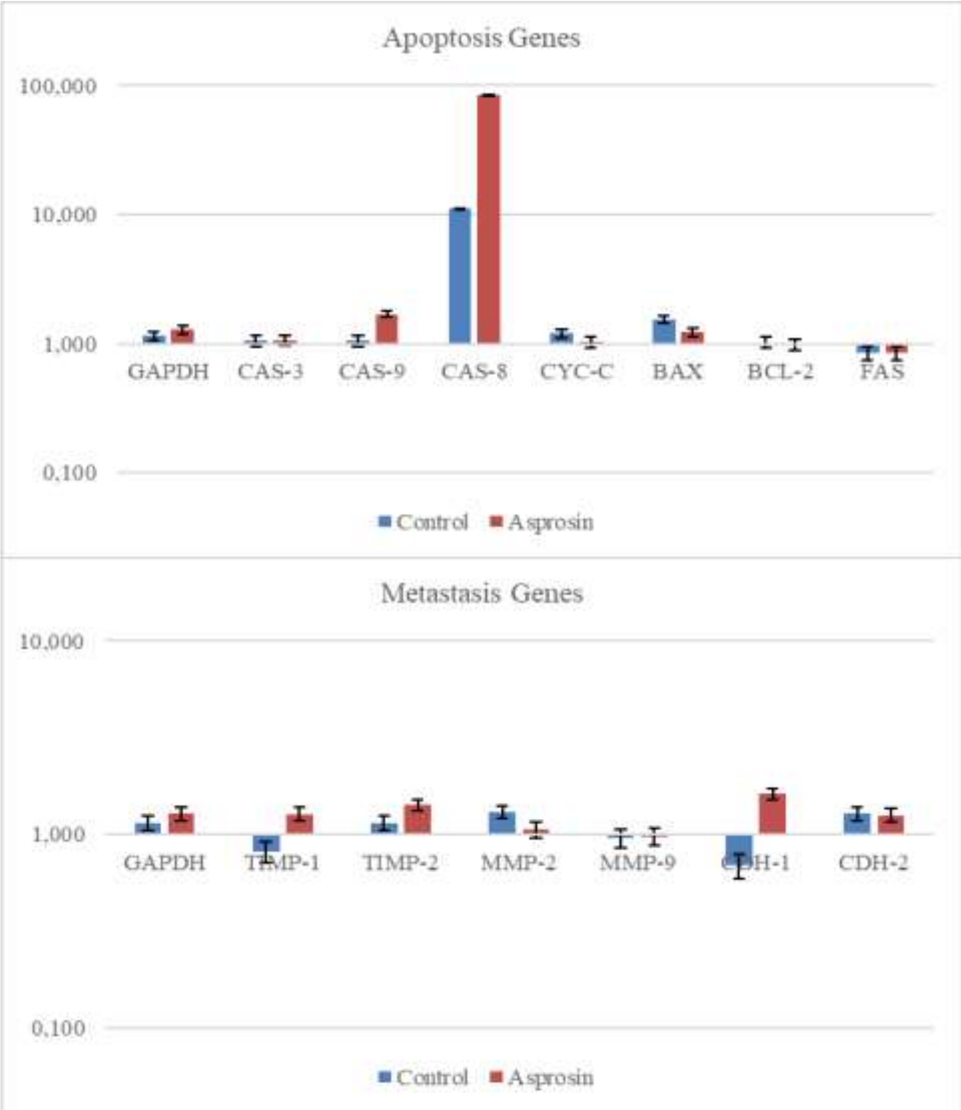


Figure 3. The results for apoptosis and metastasis genes.

Kocaman et al. discovered increased expression and positivity of asprosin when paraffin-embedded samples of the rare surface tumor known as malignant mesothelioma (MM) were compared to reactive mesothelial hyperplasia (RMH) samples as a control group (Kocaman and Artaş, 2020). In a subsequent study, the same author also proposed that asprosin might be used to distinguish between hair follicle-derived skin cancers known as hair follicle trichoblastoma (Kocaman et al., 2022) and basal cell carcinoma

(BCC). Trichoblastoma samples do not exhibit asprosin immunohistochemistry, while BCC samples exhibit a considerably higher level of asprosin immunoreactivity (Kocaman et al., 2022). As was already established, asprosin produces hyperinsulinemia and IR. These conditions can alter the equilibrium between antioxidants and oxidants and raise the production of Insulin-like growth factor 1 (IGF-1) (Du et al., 2021), which encourages the growth and metastasis of cancer (Ryu et al., 2014).

The Warburg effect, or aerobic glycolysis, is a role in the development of numerous malignancies, including SKMEL-30. Even in the presence of oxygen, the glycolytic pathway—which uses glucose for quick energy production and generates excess lactate—is frequently chosen over aerobic respiration and oxidative phosphorylation when dealing with excess glucose. It's common knowledge that breathing on its own can keep tumors alive. Oncogenesis begins with aberrant growth factor signaling regulation, which is a modifiable element in aerobic glycolysis. As a glucogenic hormone, asprosin is believed to play a part in this process and is a good subject for further study in the tumor microenvironment.

CONCLUSIONS

Based on the study, asprosin plays a critical role in the diagnosis, management, and prognosis of malignant malignancies. In the Melanoma cell line SKMEL-30 cells used in our investigation, asprosin hormone decreased the metastatic effects of the cells by lowering sialic acid levels; however, it had no effect on mitochondrial apoptosis.

REFERENCES

Booth A, Magnuson A, Fouts J, Foster M. Adipose tissue, obesity and adipokines: Role in carcinogenesis . Horm Mol Biol Clin Investig (2015) 21 :57–74. doi: 10.1515/hmbci-2014-0037 [PubMed] [CrossRef] [Google Scholar]

Du C, Wang C, Guan X, Li J, Du X, Xu Z, et al. Asprosin is associated with anorexia and body fat mass in cancer patients. Support Care Cancer (2021) 29 :1369–75. doi: 10.1007/s00520-020-05621-8 [PubMed] [CrossRef] [Google Scholar]

Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: An international consensus. Lancet Oncol (2011) 12 :489–95. doi: 10.1016/S1470-2045(10)70218-7 [PubMed] [CrossRef] [Google Scholar]

Hainaut P, Plymoth A. Cancer as a metabolic disease. Curr Opin Oncol (2012) 24 :56–7. doi: 10.1097/cco.0b013e32834e388a [PubMed] [CrossRef] [Google Scholar]

Jones RG, Thompson CB. Tumor suppressors and cell metabolism: A recipe for cancer growth. Genes Dev (2009) 23 :537–48. doi: 10.1101/gad.1756509 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Kerslake R, Sisu C, Panfilov S, Hall M, Khan N, Jeyaneethi J, et al. Differential regulation of genes by the glycogenic hormone asprosin in ovarian cancer. J Clin Med (2022) 11 :1–18. doi: 10.3390/jcm11195942 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Kocaman N, Artaş G. Could new adipokines, asprosin and meteorin-like, be biomarkers for malignant mesothelioma? Biotech Histochem (2020) 95 :171–5. doi: 10.1080/10520295.2019.1656344 [PubMed] [CrossRef] [Google Scholar]

Kocaman N, Yüksel EI, Demir B, Çalık I, Çiçek D. Two new biomarker candidates to distinguish basal cell carcinoma from trichoblastoma; asprosin and meteor-like peptide. Tissue Cell (2022) 76 . doi: 10.1016/j.tice.2022.101752 [PubMed] [CrossRef] [Google Scholar]

Paper A. Understanding the Warburg effect: Metabolic requirements of cell proliferation. Encycl Neurosci (2008) 324 :3828–8. doi: 10.1007/978-3-540-29678-2_5605 [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Qian J, Chen G, Zhang Y, Wu H, Zhao J, Wang L, et al. Lactic acid promotes metastatic niche formation in bone metastasis of colorectal cancer. Cell Commun Signal (2021) 19 :1–15. doi: 10.1186/s12964-020-00667-x [PMC free article] [PubMed] [CrossRef] [Google Scholar]

Ryu TY, Park J, Scherer PE. Hyperglycemia as a risk factor for cancer progression. Diabetes Metab J (2014) 38 :330–6. doi: 10.4093/dmj.2014.38.5.330 [PMC free article] [PubMed] [CrossRef] [Google Scholar].

Tisdale MJ. Cancer cachexia: Metabolic changes and clinical findings. Nutrition (1997) 13 :1–7. doi: 10.1016/S0899-9007(96)00313-9 [PubMed] [CrossRef] [Google Scholar]

Van Cutsem E, Arends J. Causes and consequences of cancer-related malnutrition. Eur J Oncol Nurs (2005) 9 :S51–S63. doi: 10.1016/j.ejon.2005.09.007 [PubMed] [CrossRef] [Google Scholar]

Wang W, Somers EB, Ross EN, Kline JB, O'Shannessy DJ, Schweizer C, et al. FCGR2A and FCGR3A genotypes are associated with farletuzumab response in patients with first-time relapsed ovarian cancer exhibiting low CA125. Cytogenet Genome Res (2017) 152 :169–79. doi: 10.1159/000481213 [PubMed] [CrossRef] [Google Scholar]

DETERMINATION OF ANTIBIOTIC RESISTANCE PROFILES OF *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM DIABETIC FOOT INFECTIONS

Zeynep ERDEM AYNUR[1], Bülent BOZDOĞAN[1,2],Bülent ERTUĞRUL[3],Gamze BAŞBÜLBÜL[1,4]

[1]Aydın Adnan Menderes University ADUREDPROM Laboratory

[2] Aydın Adnan Menderes University Faculty of Medicine, Department of Medical Microbiology [3] Aydın Adnan Menderes University Faculty of Medicine, Department of Infectious Diseases

[4] Aydın Adnan Menderes University, Faculty of Science, Department of Biology, Department of Molecular Biology

ABSTRACT

One of the important complication of diabetes is diabetic foot infections accompanied by neuropathy and vascular problems. These infections, especially caused by resistant bacteria, are among the most important etiological causes of foot amputations. *Pseudomonas aeruginosa* is the most common cause of diabetic foot infections in our country. The widespread and multidrug resistance seen in this bacterium poses a global threat. In this study, the antibiotic resistance profile of 100 *Pseudomonas aeruginosa* strains isolated from diabetic foot infections was determined by E-test method. **Materials and Methods:** In our study, a total of 100 *Pseudomonas aeruginosa* strains isolated from diabetic foot infections were used. The susceptibilities of different antibiotics (piperacillin, piperacillin+tazobactam, ceftazidime, meropenem, ticarcillin+clavulanate, cefepime, cefpirome, aztreonam, imipinem, amikacin, ciprofloxacin and tigecycline) in *Pseudomonas aeruginosa* isolates were determined using the E-test method**. Results:** While two of the *Pseudomonas aeruginosa* strains isolated from diabetic foot infections were resistant to all the tested antibiotics, all the isolates except one of the isolate were found to be intermediate or resistant to at least one antibiotic. The most common resistance rate among *Pseudomonas aeruginosa* strains evaluated within the scope of our study was Ticarcillin-Clavulanate (60%), followed by Ciprofloxacin (48%), Aztreonam (45%), Piperacillin (30%), Piperacillin-Tazobactam (28%), Ceftazidime. (22%), Imipenem and Meropenem (15%), Amikacin (13%). The least resistance was found against Cefepime (4%). Since there is no breakpoint value in the CLSI data of tigecycline and cefpirome, the resistance rate could not be determined. **Discussion and Conclusion:** *Pseudomonas aeruginosa*, which has multidrug resistance, is being isolated at increasing rates from diabetic foot infections in our country. According to previous studies, although the resistance situation was generally similar, it was observed that sensitivity to carbapenems was higher and the resistance to cefepime was very low. Monitoring

sensitivity to antibiotics according to isolation locations is important in directing empirical treatments.

INTRODUCTION

Diabetes is a significant public health issue, and the number of people affected by the disease is increasing daily (Jneid et al., 2017). Diabetes can lead to various complications such as kidney failure, heart attack, blindness, stroke, and lower extremity amputations. Diabetic foot ulcers are a major global health concern and are one of the most common conditions among diabetic patients. Diabetic foot ulcer is a primary reason for the hospitalization of diabetic patients. Approximately 25% of infected wounds do not heal, which can result in the amputation of the lower leg (Iraj et al., 2013).

Recent studies indicate that the causative agents of diabetic foot infections (DFIs) vary by country and geographic location (Hatipoğlu et al., 2014). In Europe, Gram-positive bacteria such as *Staphylococcus aureus* are predominantly isolated from diabetic foot ulcers. In temperate climate regions, including our country, as well as Asia and Africa, *Pseudomonas aeruginosa* is the most frequently encountered pathogen in these infections (Ertuğrul et al., 2017). For example, in a large case series study from India covering data from 1991 to 2008, Gram-negative agents accounted for 57.1% (with *P. aeruginosa* at 16.9%, being the most frequently detected agent), while Gram-positive agents were found at 40.6% (Ramakant et al., 2011). Similarly, a study from Kuwait reported a Gram-negative/positive ratio of 51%/32%, with *P. aeruginosa* at a high rate of 17.4% (Al Benwan et al., 2012). Studies conducted in our country also show that *P. aeruginosa* constitutes a high proportion of DFI agents, ranging from 15% to 20% (Ertuğrul et al., 2008; Ertuğrul et al., 2012; Ertuğrul et al., 2017). The most significant challenge in treating infections caused by this agent is antibiotic resistance. Another study from our country indicates that the treatment failure rate for DFIs caused by resistant agents is five times higher, amputation rates are increased, hospital stays are prolonged, and treatment costs are approximately twice as high compared to infections caused by susceptible agents (Ertuğrul et al., 2012).

As with most microorganisms, antibiotic resistance in *Pseudomonas aeruginosa* is increasing day by day. It is thought that inadequate infection control measures and inappropriate antibiotic use contribute to the rising antibiotic resistance rates in *Pseudomonas spp*.; this acquired antimicrobial resistance limits treatment options and complicates the management of infections. In the antimicrobial treatment of pseudomonal infections; aminoglycosides, antipseudomal cephalosporins, carbapenems, fluoroquinolones, beta-lactam/beta-lactamase inhibitors, monobactams, phosphonic acids, polymyxins are used (Magiorakos et al.,2012). Resistance of *P.aeruginosa* to antimicrobials may vary depending on time period, country, different geographical regions, hospitals and clinics of the same country (Avcıoğlu et al.2019, Çakmaklıoğulları ve Kuru, 2019, Shortridge et. al.,2019, Erdoğan et. al.,2021).

In this study, the antibiotic resistance profile of 100 Pseudomonas aeruginosa strains isolated from diabetic wound sites was examined according to CLSI criteria using the E-test method.

MATERIALS AND METHODS

Isolates Used:
A total of 100 *P. aeruginosa* isolates of diabetic foot ulcers origin from the collection of the Nazlı-Selim Eren Chronic Wound and Chronic Wound Infection Treatment Unit at Aydın Adnan Menderes University Hospital were used in the study.
E-Test Procedure:
P. aeruginosa isolates were cultured in Tryptic Soy Broth (TSB) and incubated at 37ºC for 18-24 hours. The density of the bacteria to be tested was adjusted to 0.5 McFarland. For this purpose, 100 µl from an overnight culture was suspended in 10 ml of 0.9% sterile saline solution (FTS), and the bacteria adjusted to a 0.5 McFarland density were spread on the surface of Mueller-Hinton agar using a sterile swab. The plates were left to dry in an incubator at 37ºC for 10-15 minutes. Following this, E-test strips containing a specific antibiotic gradient (piperacillin, piperacillin + tazobactam, ceftazidime, meropenem, ticarcillin + clavulanic acid, cefepime, aztreonam, cefpirome, imipenem, amikacin, ciprofloxacin, and tigecycline) were placed on the agar surface with sterile forceps, with two different strips per plate. The plates were incubated at 37ºC for 18-24 hours. The next day, the Minimum Inhibitory Concentration (MIC) values were determined. The MIC value was considered the point where the inhibition ellipse intersected with the scale on the strip (Joyce et al., 1992).

RESULTS

E-test strips were used in determining the antibiotic sensitivities of *Pseudomonas aeruginosa* isolates in accordance with the manufacturer's instructions. MIC results of all tested antibiotics were obtained as examples shown in the picture (Figure 1). Critical values of antibiotics whose MIC values were determined by E-test according to CLSI 2023 are given in Table 1, and the E-test results performed with 100 *P. aeruginosa* are given in Table 2.

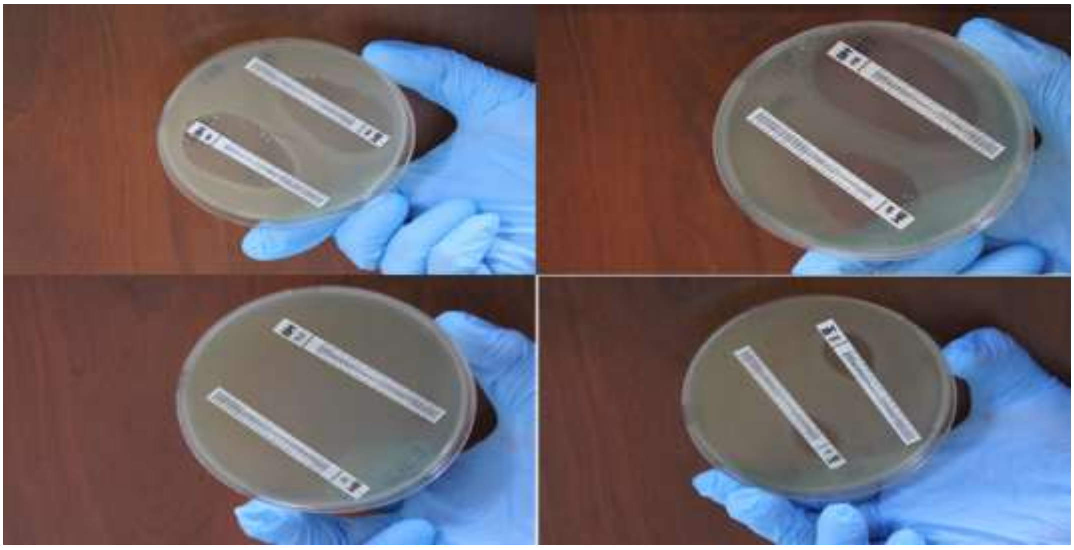


Figure 1. MIC values determined by E-test strips of different antibiotics

Table 1. Antibiotics used and their critical values (S: Sensitive, I: Intermediated R: Resistance)

Abbreviation	**Antibiotic**	**Critical values**
(TZ)	Ceftazidime	**S**≤8 **I**:16 **R**≥32
(AT)	Aztreonam	**S**≤8 **I**:16 **R**≥32
(MP)	Meropenem	**S**≤ 2 **I**:4 **R**≥8
(TGC)	Tigecycline	*There are no breakpoint values for P. aeruginosa.*
(TLC)	Ticarcillin + clavulanic acid	**S**≤16 **I**:32-64 **R**>64
(TPZ)	Piperacillin + tazobactam	**S**≤16 **I**:32-64 **R**>64
(AK)	Amikacin	**S**≤16 **I**:32 **R**≥64
(IMI)	Imipenem	**S**≤2 **I**:4 **R**≥8
(PP)	Piperacillin	**S**≤16 **I**:32-64 **R**>64
(PM)	Cefepime	**S**≤8 **I**:16 **R**≥32
(CL)	Ciprofloxacin	**S**≤0,5 **I**:1 **R**≥2
(CR)	Cefpirome	*There are no breakpoint values for P. aeruginosa..*

Table 2. E-test MIC results of *P. aeruginosa* isolates

Isolate	TZ	TLC	PP	TPZ	AT	CR	PM	MP	AK	CL	IMI	TGC
696	1.0	64.0	4.0	8.0	2.0	8.0	0.25	0.064	4.0	0.25	2.0	32.0
727	16.0	>256	8.0	16.0	>256	8.0	0.25	0.125	4.0	0.25	1.0	128.0
1039	2.0	>256	64.0	64.0	32.0	32.0	0.50	0.125	4.0	>256	0.50	128.0
2279	8.0	>256	>256	>256	128.0	>256	8.0	>256	128.0	>256	>256	128.0
337	0.5	32.0	4.0	8.0	2.0	8.0	0.25	0.32	2.0	0.25	1.0	16.0
1082	2.0	>256	8.0	16.0	>256	8.0	0.25	0.064	4.0	0.125	2.0	32.0
1668	2.0	128.0	8.0	16.0	>256	>256	1.0	0.125	4.0	>256	2.0	64.0
826	2.0	>256	32.0	16.0	4.0	8.0	0.25	0.064	4.0	4.0	0.50	16.0
526	2.0	>256	128.0	64.0	>256	>256	1.0	0.125	16.0	0.125	2.0	32.0
611	0.5	64.0	8.0	16.0	2.0	128.0	0.50	0.064	16.0	16.0	0.50	128.0
2277	2.0	>256	32.0	32.0	64.0	>256	1.0	0.064	32.0	>256	0.50	128.0
862	0.25	8.0	8.0	4.0	1.0	2.0	0.125	0.25	4.0	1.0	2.0	16.0
1930	0.5	>256	4.0	8.0	2.0	8.0	0.25	0.125	4.0	0.25	2.0	32.0
2256	2.0	>256	64.0	64.0	64.0	>256	1.0	>256	32.0	>256	1.0	>256
4468	>256	>256	>256	>256	>256	>256	8.0	16.0	64.0	>256	>256	32.0
2244	1.0	32.0	16.0	4.0	4.0	8.0	0.25	0.064	16.0	0.25	1.0	32.0
825	1.0	64.0	16.0	32.0	4.0	32.0	0.50	0.064	8.0	0.25	2.0	32.0
3940	0.50	32.0	4.0	4.0	2.0	8.0	0.125	0.032	4.0	0.25	0.25	32.0
2801	1.0	64.0	8.0	4.0	4.0	>256	>256	0.064	16.0	1.0	2.0	32.0
1666	1.0	64.0	4.0	8.0	4.0	>256	1.0	0.064	32.0	>256	2.0	128.0
3380	1.0	32.0	8.0	2.0	2.0	8.0	0.25	0.125	4.0	0.125	4.0	32.0
2597	2.0	>256	16.0	32.0	16.0	16.0	0.50	0.064	64.0	0.25	0.50	64.0
4349	>256	16.0	4.0	8.0	1.0	8.0	0.25	0.064	2.0	0.125	2.0	8.0
3590	2.0	>256	32.0	64.0	32.0	128.0	1.0	0.064	8.0	1.0	8.0	64.0
2759	128.0	128.0	64.0	32.0	128.0	>256	2.0	0.125	64.0	4.0	1.0	16.0
3150	64.0	>256	8.0	8.0	2.0	8.0	0.50	0.25	8.0	0.25	0.50	8.0
286	8.0	>256	64.0	64.0	128.0	>256	1.0	0.064	16.0	>256	1.0	64.0
1168	2.0	>256	8.0	32.0	16.0	16.0	0.25	0.25	1.0	4.0	2.0	32.0
1288	0.25	>256	4.0	4.0	1.0	0.5	0.125	0.25	2.0	0.125	1.0	8.0
2237	0.5	64.0	2.0	4.0	2.0	4.0	0.25	0.064	2.0	0.125	2.0	64.0
1104	1.0	256.0	64.0	32.0	4.0	4.0	0.50	0.25	2.0	8.0	2.0	32.0
2273	2.0	128.0	256.0	64.0	64.0	256.0	1.0	0.125	2.0	>256	16.0	32.0
2913	1.0	128.0	128.0	32.0	4.0	32.0	0.25	0.125	2.0	32.0	4.0	16.0
1834	>256	>256	32.0	128.0	32.0	64.0	0.50	0.50	2.0	>256	1.0	32.0
1885	1.0	>256	128.0	>256	256.0	>256	0.50	1.0	4.0	>256	0.50	16.0
3787	128.0	1.0	32.0	8.0	16.0	1.0	0.125	0.25	0.50	0.032	0.50	0.50
2711	4.0	>256	256.0	256.0	>256	>256	2.0	>256	8.0	8.0	>256	32.0
399	0.50	32.0	8.0	8.0	4.0	4.0	0.25	0.125	2.0	0.125	2.0	16.0
564	0.50	32.0	8.0	8.0	8.0	64.0	0.25	0.125	2.0	0.64	2.0	32.0
1444	1.0	>256	64.0	256.0	32.0	64.0	0.50	0.125	2.0	>256	1.0	64.0
1897	1.0	128.0	8.0	8.0	8.0	4.0	0.50	0.064	8.0	0.25	0.50	32.0

2971	>256	>256	>256	>256	>256	>256	8.0	0.125	2.0	0.125	1.0	16.0
390	2.0	>256	>256	16.0	16.0	>256	1.0	1.0	8.0	0.50	2.0	32.0
661	0.50	256.0	8.0	32.0	8.0	16.0	0.25	0.25	2.0	0.50	1.0	32.0
K1	0.50	32.0	4.0	2.0	2.0	4.0	0.25	0.125	2.0	0.125	1.0	16.0
K2	1.0	32.0	4.0	4.0	1.0	8.0	0.25	0.125	2.0	0.125	0.25	64.0
K3	2.0	>256	>256	>256	128.0	>256	2.0	32.0	32.0	>256	>256	128.0
K4	0.50	64.0	2.0	2.0	4.0	2.0	0.25	0.125	2.0	0.25	2.0	16.0
K5	2.0	128.0	256.0	64.0	64.0	32.0	0.50	0.064	4.0	2.0	2.0	64.0
K6	1.0	16.0	4.0	8.0	4.0	8.0	0.25	0.125	2.0	8.0	1.0	32.0
K7	1.0	64.0	8.0	8.0	8.0	16.0	4.0	0.125	8.0	0.25	2.0	16.0
K8	16.0	64.0	32.0	32.0	0.25	2.0	1.0	0.064	4.0	8.0	>256	128.0
K9	4.0	>256	32.0	64.0	>256	32.0	0.50	1.0	4.0	1.0	0.50	32.0
K10	2.0	>256	>256	32.0	32.0	>256	1.0	1.0	8.0	0.25	2.0	32.0
K11	>256	>256	>256	>256	64.0	>256	1.0	4.0	4.0	0.125	4.0	32.0
K12	32.0	>256	>256	>256	>256	>256	2.0	>256	64.0	>256	4.0	16.0
952	0.50	48.0	8.0	8.0	4.0	8.0	0.25	0.125	4.0	0.64	4.0	16.0
1038	0.50	48.0	8.0	8.0	4.0	8.0	0.25	0.125	4.0	0.64	4.0	16.0
1337	2.0	8.0	4.0	4.0	8.0	0.50	1.0	0.50	2.0	16.0	1.0	32.0
1275.3	>256	>256	256.0	128.0	8.0	>256	2.0	0.50	128.0	>256	2.0	32.0
1040	32.0	>256	>256	>256	256.0	>256	1.0	16.0	4.0	0.125	4.0	16.0
2777	0.50	>256	32.0	32.0	64.0	16.0	0.25	0.064	2.0	0.25	1.0	32.0
2951	1.0	>256	>256	128.0	64.0	32.0	0.50	0.25	2.0	2.0	1.0	32.0
3079	0.50	16.0	2.0	4.0	4.0	32.0	0.125	0.064	2.0	0.50	4.0	32.0
3084	4.0	>256	>256	>256	>256	>256	32.0	>256	>256	>256	>256	64.0
3121	0.50	32.0	4.0	2.0	4.0	16.0	0.25	0.50	2.0	0.125	1.0	16.0
3228	1.0	>256	64.0	128.0	256.0	128.0	0.50	>256	2.0	>256	16.0	32.0
2241	>256	>256	>256	>256	>256	>256	8.0	8.0	32.0	>256	4.0	64.0
2243	1.0	128.0	16.0	16.0	32.0	>256	1.0	0.25	8.0	2.0	2.0	64.0
1871	1.0	32.0	2.0	4.0	2.0	>256	1.0	0.125	32.0	>256	1.0	64.0
1092	>256	>256	>256	128.0	>256	>256	4.0	0.50	64.0	>256	2.0	32.0
127C	>256	>256	>256	128.0	>256	>256	4.0	0.50	64.0	>256	2.0	32.0
3362	0.50	32.0	4.0	32.0	4.0	16.0	0.25	0.25	8.0	0.25	2.0	32.0
3420	0.50	32.0	4.0	32.0	4.0	16.0	0.25	0.125	4.0	0.125	2.0	32.0

Isolate	TZ	TLC	PP	TPZ	AT	CR	PM	MP	AK	CL	IMI	TGC
3360	1.0	32.0	4.0	8.0	4.0	16.0	0.50	0.064	2.0	0.25	4.0	32.0
3504	1.0	>256	4.0	32.0	8.0	16.0	0.5	2.0	2.0	0.125	2.0	64.0
3583	>256	>256	>256	>256	>256	>256	128.0	>256	>256	>256	>256	32.0
1126	1.0	32.0	4.0	4.0	2.0	8.0	0.25	0.125	4.0	0.25	2.0	64.0
4631	1.0	32.0	4.0	4.0	2.0	8.0	0.25	0.125	4.0	0.25	2.0	64.0
4201	128.0	>256	>256	>256	>256	>256	2.0	0.25	8.0	32.0	2.0	32.0
4329	1.0	>256	32.0	32.0	64.0	64.0	0.25	0.125	2.0	32.0	2.0	128.0
4357	0.75	>256	16.0	32.0	32.0	32.0	0.25	0.125	2.0	2.0	2.0	16.0

4685	64.0	>256	>256	>256	256.0	>256	2.0	1.0	4.0	4.0	1.0	8.0
4629	>256	>256	>256	>256	>256	>256	8.0	4.0	4.0	2.0	32.0	8.0
4519	0.25	2.0	0.50	0.25	0.125	0.064	0.125	0.064	2.0	0.064	1.0	4.0
4696	>256	>256	>256	>256	>256	>256	8.0	2.0	32.0	>256	2.0	32.0
4730	1.0	32.0	>256	>256	>256	>256	2.0	>256	4.0	>256	>256	4.0
4710	>256	>256	>256	>256	>256	>256	64.0	>256	>256	>256	>256	32.0
4759	>256	>256	>256	>256	>256	>256	2.0	2.0	64.0	>256	1.0	32.0
5037	4.0	>256	32.0	>256	64.0	256.0	0.50	0.25	4.0	>256	1.0	64.0
5062	1.0	128.0	8.0	16.0	4.0	>256	0.50	0.25	2.0	0.50	0.50	32.0
5012	1.0	64.0	4.0	8.0	2.0	16.0	0.50	0.25	4.0	0.125	2.0	8.0
5197	0.50	32.0	4.0	8.0	8.0	8.0	0.25	0.064	4.0	0.125	2.0	16.0
5193	1.0	64.0	4.0	8.0	2.0	32.0	0.25	0.125	8.0	0.25	4.0	32.0
5103	1.0	256.0	16.0	32.0	8.0	>256	0.50	32.0	16.0	>256	>256	32.0
5102	32.0	16.0	>256	128.0	>256	>256	4.0	0.25	16.0	2.0	4.0	1.0
5198	1.0	>256	32.0	32.0	16.0	>256	0.50	32.0	16.0	>256	>256	>256
5199	0.50	32.0	4.0	4.0	8.0	16.0	0.25	0.064	4.0	0.125	2.0	16.0
5360	0.50	32.0	16.0	32.0	16.0	4.0	0.50	0.064	8.0	0.125	2.0	4.0
5785	0.5	>256	4.0	8.0	2.0	8.0	0.25	0.125	4.0	0.25	2.0	32.0

While two of the *Pseudomonas aeruginosa* strains isolated from diabetic foot infections were resistant to all tested antibiotics, all isolates except one were found to be intermediate or resistant to at least one antibiotic. The highest resistance rates among *P. aeruginosa* isolates were, Ticarcillin and Clavulanic acid (60%) respectively, It was determined as Ciprofloxacin (48%) and Aztreonam (45%). The highest sensitivity rates were found for the antibiotics cefepime (96%), meropenem (83%) and amikacin (81%), respectively (Table 3)

Table 3. Antibiotic sensitivity/resistance rates according to MIC results

Antibiotic	Rate of sensitive isolates (%)	Rate of intermediate isolates (%)	Rate of resistant isolates (%)
TZ	76	2	22
AT	49	6	**45**
MP	**83**	2	15
TGC	*There are no breakpoint values for P. aeruginosa*		
TLC	9	31	**60**
TPZ	45	27	28
AK	**81**	6	13
PP	52	18	30
PM	**96**	0	4
CL	4	48	**48**
IMI	73	12	15
CR	*There are no breakpoint values for P. aeruginosa*		

DISCUSSION

Diabetes, with its rising prevalence and incidence, is considered as a global health problem, currently affecting around 171 million people worldwide and projected to reach 366 million by 2030 (Pedras et al.,2016). Diabetic patients have a higher rate of developing foot wound infections than non-diabetic individuals due to reasons such as deterioration of microvascular circulation, neuropathy, deterioration in immune capacity and anatomical changes. Therefore, one of the important complications of diabetes mellitus is diabetic foot ulcers (Abdulrazak et al.,2005). Diabetic neuropathy and peripheral arterial disease (PAD) play an important role in the development of foot ulcers complicated by infection and are also considered a strong indicator of amputation. Additionally, the high recurrence rate of diabetic foot ulcers, due to immune suppression and treatment costs ranging from $900 to $4,595 per episode in different countries, not only hampers the quality of life but also imposes a significant financial burden on patients (Mairghani et al.,2019).

It has been reported that over 50% of people with diabetic foot ulcers become infected by various types of microorganisms due to the damage to the protective skin layer. (Lipsky et al.,2012). Potential causative organisms of diabetic foot ulcers (DFUs) are believed to include *Staphylococcus, Streptococcus, Proteobacteria, Pseudomonas* *aeruginosa,* and coliform bacteria (Lipsky et al.,2015). Numerous studies have shown that *Staphylococcus aureus* is the predominant pathogen of diabetic foot ulcers in Western countries, while *Pseudomonas* is more common in Asian and African countries (Kwon and Armstrong,2018). Empiric antibiotic therapy is a crucial aspect of managing diabetic foot ulcers (DFUs). In recent years, the use of antimicrobial agents has become challenging due to the emergence of multi-drug resistant (MDR) bacteria, making the selection of appropriate antibiotics difficult. Antibiotic resistance rates have been increasing at an alarming rate in low- and middle-income countries. The rate of diabetic foot ulcers is increasing in developing countries due to inadequate knowledge about foot care among diabetic patients, an inefficient primary health care system, and low socio-economic status (Al-Rubeaan et al., 2015, Sharoni et al., 2017)

Aminoglycosides have a concentration-dependent bactericidal effect and are among the oldest antibiotics used in the treatment of infections caused by Gram-negative and some Gram-positive bacteria. While the amikacin resistance rate in *P. aeruginosa* varies between 0-29.5% in national studies, this rate is between 13.5-57% in international studies (Kara et al.,2014, Du et al.,2022, Öner et al.,2022, Makeri et al.,2023). The 13% resistance rate we obtained in our study is within the Türkiye and world average.

Ceftazidime, a third-generation cephalosporin, and cefepime, a fourth-generation cephalosporin, are antimicrobials with antipseudomal activity. In national studies, the cefepime resistance rate was reported as 24.2-42.3% and the ceftazidime resistance rate was reported as 11- 58,3 % (Aykan and Çiftçi, 2015, Avcıoğlu et al.,2019, Erdoğan et al.,2021). When *the P. aeruginosa* resistance data of the SENTRY antimicrobial surveillance program, which includes the Asia-Pacific region, Europe, Latin America and North America, was evaluated between 1997 and 2016, the cefepime resistance rate was reported as 20.7% and the ceftazidime resistance rate was reported as 22.5% (Shortridge et al.,2021). In the meta-analysis study conducted by Du et al. in China, the ceftazidime resistance rate was 33.9%, the cefepime resistance rate was 20% (Du et al., 2022). In meta-analysis studies conducted in Africa, resistance rates were found to be 48.48% for ceftazidime and 22% for cefepime (Makeri et al., 2023, Wada et al., 2023). Cefpirome, a broad-spectrum cephalosporin tested in our study, does not have a breakpoint value for *Pseudomonas aeruginosa* in CLSI data. *Pseudomonas aeruginosa* can develop resistance mechanisms to various antibiotics, especially to broad-spectrum cephalosporins such as cefpirome.

Ciprofloxacin, a member of the fluoroquinolone class of antibiotics, has been used successfully to treat a wide range of bacterial infections since 1987 and is included in the World Health Organization's list of essential medicines (Rehman et al.2018). Ciprofloxacin allows oral or intravenously, and recently an inhalable treatment of many infections that require parenteral treatment due to its bioavailability, good penetration into tissue, and low side effects (Şenol,2002, Kłodzińska et al.,2016). In national studies, the ciprofloxacin resistance rate has been reported as 7-48% (Gönüllü et. al., 2003, Gültekin et al.,2004). According to the results of meta-analysis conducted in China, Africa and

Iran, ciprofloxacin resistance in *Pseudomonas aeruginosa* was determined as 37.5%, 24.58% and 19.6%, respectively. (Shahrakh et al.,2021, Du et al.,2022, Wada et al.,2023). In our study, the ciprofloxacin resistance rate was found to be 48%. The resistance rate was higher than that mentioned studies .

The increasing level of carbapenem (imipenem/meropenem) resistance in *Pseudomonas aeruginosa* is an important public health problem. Resistance significantly limits treatment options. Significant differences are observed in the percentages of carbapenem-resistant *P. aeruginosa* across the WHO European Region. While resistance development of 5% or less was detected in only two of the countries where Central Asian and European Surveillance of Antimicrobial Resistance data were obtained, carbapenem resistance was found to be over 50% in 14 countries. Again, according to these data, the carbapenem resistance rate in Turkey is between 25-50% (WEB1, 2024). In our study, the carbapenem resistance rate was found to be 15% for both imipenem and meropenem, which showed lower percentage of resistance in comparison to other studies in Turkey and in many other countries..

Piperacillin extends the spectrum of activity of ampicillin to include most strains of *Pseudomonas aeruginosa*, *Enterobacteriaceae* (non-β-lactamase producing), many *Bacteroides* species, and *Escherichia faecalis*. (Pasifici,2023). In a study conducted in our country, the resistance rate to piperacillin was reported as 42.8%. According to the results of the meta-analysis conducted in China, piperacillin resistance rate was found to be 23,1% (Du et al., 2022).

The production of β-lactamases is the most common mechanism by which Gram-negative bacteria develop resistance to β-lactam antibiotics. A successful method to circumvent the threat of plasmid-encoded β-lactamases is to combine penicillin with inhibitors of these enzymes. Ticarcillin-clavulanate and piperacillin-tazobactam have the broadest spectra of activity, including effectiveness against *Pseudomonas aeruginosa*. Although ticarcillin-clavulanate and piperacillin-tazobactam have similar spectra of activity, they exhibit many differences. (Lister, 2000). In various studies conducted in our country, the resistance rate to ticarcillin clavulanate and piperacillin tazobactam in *Pseudomonas aeruginosa* was found to be approximately 38%. According to the results of the meta-analysis conducted in China, piperacillin resistance rate was found to be 15,4% (Du et al., 2022) Piperacillin/tazobactam, which is formed when combined with the β-lactamase inhibitor tazobactam, has a much broader antibacterial spectrum (Pasifici,2023).) In a study covering Western Europe, the piperacillin-tazobactam resistance rate was found to be 25,2%. In the same study, this resistance rate was found to be 88.9% in *Pseudomonas aeruginosa* with multidrug resistance (Karlowsky et al., 2023). In our study, the ticarcillin clavulanate resistance rate was found to be 60% and the piperacillin/tazobactam resistance rate was 28%. In our study, the resistance rate developing in piperacillin tazobactam was at a level similar to the studies conducted in Turkey and the world, while the resistance rate in ticarcillin clavulanate was found to be quite high.

Aztreonam is the first monobactam drug to enter antibacterial therapy. It is completely synthetic. It has a relatively narrow spectrum. It can be used instead of aminoglycosides in Gram (-) bacterial infections. It is an antibiotic that has not been used in the treatment of *Pseudomonas aeruginosa* in our country in recent years. The *Pseudomonas aeruginosa* aztreonam resistance rate in our country was found to be 49.6%. In a study covering France, Germany, Italy, Portugal, Spain and the United Kingdom, aztreonam resistance was found to be 19.1%, and in the examination of *Pseudomonas* with multidrug resistance in the same countries, the resistance rate was found to be 73.1% (Karlowsky et al., 2023). In meta-analysis studies conducted in China, the aztreonam resistance rate in *Pseudomonas* was found to be 45.2% (Du et. al.,2022). The 45% resistance rate we found in our study is within the Türkiye average. It is thought that the reason why aztreonam resistance is so common may be due to other antibiotic groups that can induce the synthesis of plasmid-controlled extended-spectrum β-lactamase (ESBL) and chromosomal inducible β-lactamase (IBL) seen in *Pseudomonas* species and are in active use in treatment (Baddal et.al.,2021).

Studies have shown that the majority of *Pseudomonas aeruginosa* strains exhibit natural resistance to tigecycline, making its use as a monotherapy in treating *Pseudomonas* infections limited (Zhanel et al., 2008). In another study conducted by Biedenbach and colleagues, the activity of tigecycline against pathogens isolated from patients with skin infections was examined, and it was found that *Pseudomonas aeruginosa* isolates exhibited high resistance to tigecycline (Biedenbach et al.,2005). In our study, evaluation could not be performed for tigecycline against *Pseudomonas aeruginosa* isolates due to the absence of a breakpoint value in the CLSI data. Among the 100 isolates evaluated, a MIC value of 256 or higher was detected in 52 isolates.

The resistance rates we observed in our study are generally consistent with those found in other similar studies conducted in our country. However, contrary to the general situation, we found a higher sensitivity to carbapenems and a significantly lower resistance to cefepime. Differences in antibiotic susceptibility results among hospitals vary depending on antibiotic use policies, the clinic where the patients from whom the strains were isolated were hospitalized, and the patients' underlying diseases. The observed differences in antibiotic resistance patterns highlight the need for ongoing surveillance and collection of localized resistance data to guide effective treatment strategies.

REFERENCES

Abdulrazak A, Bitar ZI, Al-Shamali AA, Mobasher LA. Bacteriological study of diabetic foot infections. J Diabetes Complicat 2005;19(3):138-41.

Al Benwan K, Al Mulla A, Rotimi VO. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J Infect Public Health 2012;5(1):1-8.

Avcıoğlu F, Karabörk Ş, Kurtoğlu MG, Behçet M. Çeşitli klinik örneklerden izole edilen Pseudomonas aeruginosa suşlarının antimikrobiyal direnç oranları: üç yıllık değerlendirme. ANKEM Derg. 2019;33(2):43-8.

Aykan ŞB, Çiftci İH. Meta-Analiz: Türkiye'de Pseudomonas aeruginosa izolatlarının son 11 yıldaki antibiyotik direnç değişimi. Mikrobiyol Bul. 2015;49(3):352-65.

Baddal B., Güler E., Süer K. Hastane ve Toplum Kökenli İnfeksiyonlardan İzole Edilen Pseudomonas aeruginosa Suşlarında Antibiyotik Direnci: Kuzey Kıbrıs'ta Güncel Durum. Klimik Dergisi 2021; 34:31-6

Biedenbach DJ, Beach ML, Jones RN. Activity of Tigecycline and Comparators against Pathogens Isolated from Patients with Skin and Skin-Structure Infections. Diagnostic Microbiology and Infectious Disease.2005

Çakmaklıoğulları EK, Kuru C. Pseudomonas aeruginosa suşlarının antibiyotik duyarlılıkları: Farklı örnek türlerinde değerlendirme. ANKEM Derg. 2019;33(2):37-42.

Du F, Ma J, Gong H, Bista R, Zha P, Ren Y, Gao Y, Chen D, Ran X and Wang C (2022) Microbial Infection and Antibiotic Susceptibility of Diabetic Foot Ulcer in China: Literature Review. Front. Endocrinol. 13:881659. doi: 10.3389/fendo.2022.881659

Erdoğan MM, Acun Delen L, Erdoğan E. Yoğun bakım ünitelerinden izole edilen Pseudomonas aeruginosa suşlarının antibiyotiklere duyarlılıkları. İnönü Üniversitesi Sağlık Hizmetleri Meslek Yüksek Okulu Derg. 2021;9(1):230-37.

Ertuğrul, M.B., Baktiroglu, S., Salman, S., Unal, S., Aksoy, M., Berberoglu, K., Calangu, S. 2008. "Pathogens isolated from deep soft tissue and bone in patients with diabetic foot infections", Journal of American Pediatric Medicine Association 98 (4), 290-295.

Ertuğrul, B,M,, Oncul, O., Tulek, N., Willke, A., Sacar, S., Tunccan, O.G., Yilmaz E., Kaya, O., Ozturk, B., Turhan, O. et al. 2012. "A prospective, multi-center study: factors related to the management of diabetic foot infections", European Journal of Clinical Microbiology and Infectious Diseases, 31 (9), 2345-2352.

Ertuğrul, M.B., Uyar-Güleç, G., Baktıroğlu, S., Çörekli E., Türe, M. 2017. "Diyabetik ayak infeksiyonu etkenlerinin yıllara göre dağılımı: Değişim var mı? Klimik Dergisi, 30, 27 - 31.

Gönüllü N, Gürol Y, Bülüç M, Bal Ç. Pseudomonas aeruginosa suşlarında görülen beta-laktam direnç fenotipleri ve antibiyotik duyarlılıkları. Hastane İnfeksiyonları Dergisi 2003; 7: 141-7.

Gültekin B, Eyigör M, Aydın N. Klinik örneklerden izole edilen Pseudomonas kökenlerinin antibiyotik direnci. ANKEM Derg 2004; 18 : 1-4.

Hatipoglu, M., Mutluoglu, M., Uzun, G., Karabacak, E., Turhan, V., Lipsky, B.A. 2014. "The microbiologic profile of diabetic foot infections in Turkey: a 20-year systematic review: diabetic foot infections in Turkey", European Journal of Clinical Microbiolology and Infectous Diseases, 33 (6), 871-878.

Iraj B., Khorvash F., Ebneshahidi A., Askari G. Prevention of diabetic foot ulcer. Int J Prev Med. 2013 Mar;4(3):373-6.

Jneid J., Cassir N., Schuldiner S., Jourdan N., Sotto A., Lavigne J.P. and La Scola B. Exploring the Microbiota of Diabetic Foot Infections With Culturomics. Front. Cell. Infect. Microbiol. 2018, 8:282. doi: 10.3389/fcimb.2018.00282

Joyce L.F., Downes J., Stockman K., and Andrew J H. Comparison of five methods, including the PDM Epsilometer test (E test), for antimicrobial susceptibility testing of Pseudomonas aeruginosa. J Clin Microbiol. 1992 Oct; 30(10): 2709–2713.
Kara Z.,Örmen B. Türker N., Vardar i., Ural S., El S., Kaptan F., Demirdal F. Diyabetik Ayak İnfeksiyonlarının Klinik ve Bakteriyolojik Olarak Değerlendirilmesi. Klimik Dergisi 2014; 27(1): 21-5
Karlowsky J.A., Loba SH, Siddiquic F., Akrichd, B., DeRyke CA., Young K., Motyl MR., Hawser SP., Sahm DF. In vitro activity of ceftolozane/tazobactam against multidrug-resistant Pseudomonas aeruginosa from patients in Western Europe: SMART 2017-2020. International Journal of Antimicrobial Agents 61 (2023) 106772
Khalid Al-Rubeaan ,Mohammad Al Derwish,Samir Ouizi,Amira M. Youssef,Shazia N. Subhani, Ibrahim HM., Alamri BN. Diabetic foot complications and their risk factors from an large retrospective cohort study. PLoS One 2015;10(5):e0124446.
Kłodzińska SN, Priemel PA, Rades T, Mørck Nielsen H. Inhalable antimicrobials for treatment of bacterial biofilm-associated sinusitis in cystic fibrosis patients: challenges and drug delivery approaches. Int J Mol Sci 2016; 17:1688
Kwon KT. and Armstrong DG. Microbiology and antimicrobial therapy for diabetic foot infections. Infection & chemotherapy 2018;50(1):11
Lipsky BA, Berendt AR, Cornia PB., Pile JC, Peters EJG., Armstrong DG., Deery HG, Embil JM., Joseph WS., Karchmer AW., Pinzur MS., Senneville E. Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. 2012 Clin Infect Dis 2012;54(12):e132e73.
Lipsky BA., Aragón-Sánchez J.,Diggle M., Embil J., Kono S., Lavery L.,Senneville E., Urbančič-Rovan V., Van-Asten S., Peters EJG., International Working Group on the Diabetic Foot. IWGDF guidance on the diagnosis and management of foot infections in persons with diabetes. Diabetes Metab Res Rev 2016;32(Suppl1):45e74.
Lister PD. β-Lactamase Inhibitor Combinations with Extended-Spectrum Penicillins: Factors Influencing Antibacterial Activity against Enterobacteriaceae and Pseudomonas aeruginosa. Pharmacotherapy 2000;20(9 Pt 2):213S–218S
Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.
Mairghani M, Jassim G., Elmusharaf K.,Patton D., Eltahir O., Moore Z., Sorensen J. Methodological approaches for assessing the cost of diabetic foot ulcers: a systematic literature review. J Wound Care 2019;28(5):261e6.
Makeri D. , Odoki M., Eilu E. 1 and Agwu E. Update on prevalence and antimicrobial resistance of Staphylococcus aureus and Pseudomonas aeruginosa isolated from diabetic foot ulcers in Africa: a systematic review and meta-analysis Bulletin of the National Research Centre (2023) 47:145https://doi.org/10.1186/s42269-023-01119-5
Öner SZ., Kaleli İ., Demir M., Mete E., Çalışkan A., Ergin Ç. PSEUDOMONAS AERUGINOSA İZOLATLARININ ANTİBİYOTİK DİRENCİ VE YILLAR İÇİNDEKİ DEĞİŞİMİ ANKEM Derg 2022;36(1):9-15. doi: 10.54962/ankemderg.1107814

Pacifici GM. Clinical Pharmacology of Piperacillin/Tazobactam. J Clin Biomed Invest 2023, 3:1

Pedras S, Carvalho R, Pereira MG. Quality of life in Portuguese patients with diabetic foot ulcer before and after an amputation surgery. Int J Behav Med 2016;23:714e21.

Ramakant, P., Verma, A.K, Misra, R., Prasad, K.N., Chand, G., Mishra, A., Agarwal, G., Agarwal, A., Mishra, S.K., 2011. "Changing microbiological profile of pathogenic bacteria in diabetic foot infections: time for a rethink on which empirical therapy to choose?" Diabetologia, 54 (1),58-64.

Sharoni SKA., Abdul Rahman H., Minhat HS:, Ghazali SS., Ong MHA.. A self-efficacy education programme on foot self-care behaviour among older patients with diabetes in a public long-term care institution, Malaysia: a Quasi-experimental Pilot Study. BMJ Open 2017;7(6):
e014393.

Shortridge D, Gales AC, Streit JM, Huband MD, Tsakris A, Jones RN. Geographic and temporal patterns of antimicrobial resistance in Pseudomonas aeruginosa over 20 years from the SENTRY antimicrobial surveillance program, 1997–2016. In Open Forum Infectious Diseases, 6. baskı, No. 1, s.63-8, Oxford University, US (2019).

Şenol E. Siprofloksasin. ANKEM Derg. 2002;16(3):382-84.

Wada F.W, Mekonnen M.F., Sawiso E.D.,Kolato S., Woldegiorgis L., Kera G.K., El-Khatib Z. Ashuro A.A.1, Biru M.and Boltena M.T. Bacterial profile and antimicrobial resistance patterns of infected diabetic foot ulcers in sub-Saharan Africa: a systematic review and meta-analysis. Scientific Reports | (2023) 13:14655 | https://doi.org/10.1038/s41598-023-41882-z

WEB1. https://www.who.int/europe/publications/i/item/9789289058537 Antimicrobial resistance surveillance in Europe 2023–2021 data. Erişim Tarihi: 28/06/2024

Zhanel GG, DeCorby M, Laing N, et al. In Vitro Activity of Tigecycline against Multidrug-Resistant Pseudomonas aeruginosa. Journal of Antimicrobial Chemotherapy. 2008

EVALUATION OF PHYTOCHEMICAL COMPONENTS OF ACHILLEA MILLEFOLIUM IN DIFFERENT AREAS OF ALBANIA USING SUPERCRITICAL CO_2 EXTRACTION

Edlira Kaloshi[1], Lirika KUPE[2], Kleva Shpati[1], Aida Dama[1] Marsela Alikaj[2]

[1]Albanian University, Faculty of Medical Sciences, Department of Pharmacy.
[2]Agricultural University of Tirana, Faculty of Agriculture and Environment, Department of Agronomic Sciences

Corresponding author e-mail: edlirahe@yahoo.co.uk

ABSTRACT

Achillea millefolium, commonly known as yarrow, is a perennial herb that belongs to the Asteraceae family. *A. millefolium* continues to be valued in modern herbal medicine for its broad spectrum of therapeutic applications, rooted in centuries-old traditional knowledge and validated by contemporary scientific research. The medicinal properties of *A. millefolium* are attributed to its complex phytochemical composition, which includes various classes of bioactive compounds. Essential oil of *A. millefolium* contains various compounds such as mono- and sesquiterpenoids, including pinenes, sabinene, phellandrene, terpinen-4-ol, alpha-terpineol, β-caryophyllene, bornyl acetate, azulene. These components contribute to the diverse pharmacological activities exhibited by *A. millefolium*. The objective of this study is to evaluate and identify the chemical components present in *A. millefolium* using supercritical CO_2 extraction.
The plant material was collected in May-July 2023 from 4 various regions in Albania (Elbasani, Librazhdi, Pogradeci, Korca) and then subjected to supercritical CO_2 extraction. The extracted compounds were analyzed using flame ionization gas chromatograph. The most abundant chemical components were 1.8-Cineole (6.2%-8.08%), b-Caryophyllene (19.8%- 24%), Germancene (11%-12.4%), Bornyl acetate (4.8%-6%), b-Pinene (6.2%-7.1%).

Keywords: *Achillea millefolium*, essential oil, supercritical CO_2 extraction.

INTRODUCTION

Achillea millefolium, commonly known as yarrow, is a perennial herb belonging to the Asteraceae family. It is found throughout Albania, from the northernmost to southernmost regions. This herbaceous plant has an unbranched stem that grows up to 80 cm tall and is characterized by its white, umbrella-shaped flowers and green to grey leaves. It is lightly aromatic with a bitter taste and blooms from June to November. *A. millefolium* is prevalent in meadows and mountainous areas, sometimes even in forests.

It is found at elevations starting from 500-600m above sea level and can reach up to 2400 m above sea level in mountain peaks. This plant has a long history of traditional medicinal use across various regions (Hassanzadeh-Kiabi & Negahdari, 2017). The medicinal properties of *A. millefolium* are attributed to its complex phytochemical composition, which includes various classes of bioactive compounds. Essential oil of *A. millefolium* contains various compounds such as mono- and sesquiterpenoids, including pinenes, sabinene, phellandrene, terpinene-4-ol, alpha-terpineol, β-caryophyllene, bornyl acetate, azulene. This herb has been utilized in traditional medicine for its anti-inflammatory, antioxidant, and immunological properties (Al-Ezzy et al., 2018). Studies have shown that A. millefolium exhibits a wide range of pharmacological effects, including anti-inflammatory, antipyretic, analgesic, and antioxidant activities (El-Sadek et al., 2007). Additionally, research has highlighted its potential as an antimicrobial agent against antibiotic-resistant bacteria (Aliasghari et al., 2017). The phytochemical composition of Achillea millefolium has been extensively studied, revealing the presence of bioactive components such as phenolic compounds with significant bioactivity (Vitalini et al., 2011). These compounds contribute to the herb's pharmacological profile, making it a valuable resource in herbal medicine (Satari et al., 2021). Furthermore, *A. millefolium* has been investigated for its potential to alleviate testicular damage, with studies demonstrating its ability to mitigate testicular oxidative stress and inflammatory responses (Okkay et al., 2021).

Moreover, *A. millefolium* has been explored for its effects on various health conditions, such as its use in mouthwash to prevent and treat oral mucositis induced by chemotherapy (Hajisalem et al., 2019). It has also shown promising effects in improving blood glucose levels, liver enzymes, and lipid profiles in diabetic rats (Rezaei et al., 2020).

Additionally, the herb has been associated with blood pressure-lowering, cardiovascular inhibitory, and bronchodilators actions, supporting its traditional use in treating cardiovascular and respiratory conditions (Khan & Gilani, 2010). The plant has also been investigated for its hepatoprotective effects and its ability to stimulate human skin fibroblast cells (Alzomor et al., 2022; Ghobadian et al., 2015) In recent years, numerous studies have been conducted in the field of nanotechnology utilizing *A. millefolium* as a component of nanoparticles. The results have shown promising effects of *A. millefolium* formulations as a practical distribution system. In conclusion, *Achillea millefolium* stands out as a versatile herb with a rich pharmacological profile, offering a wide array of potential health benefits. Its traditional uses are supported by modern scientific research, highlighting its importance in herbal medicine and pharmaceutical applications. The objective of this study is to evaluate and identify the chemical components present in *A. millefolium* in different regions of Albania using supercritical CO_2 extraction, as a suitable method for extracting valuable oils and some organic compounds from plants and explaining the pharmacological importance of the chemical components extracted from this plant. In other study the evaluation of the chemical composition of *A. millefolium* essential oil in Albania (in four different sampling sites: Kukësi, Tirana, Librazhdi and Përmeti) was conducted by the hydro distillation method and gas chromatography with a flame ionization detector. Evaluating borneol as the most noteworthy compound, with a percentage range of 19.62% to 20.72%. Cineole follows, with values ranging from 16.32% to 24.33%, succeeded by Azulenes with values ranging from 3.19% to 18.83%, (Kaloshi et al., 2023)

Supercritical-CO_2 (SC-CO_2) extraction is a suitable method for extracting valuable oils and some organic compounds from plants (Argun 2022). Carbon dioxide (CO_2) is used under supercritical conditions to extract the principal aromatics from plant materials without using organic solvents (DURAK et al., 2023). Supercritical fluid extraction (SFE) was developed for analytical application in the mid-1980s to reduce the use of organic solvents in the laboratory environment and is now a standard method for the extraction, fractionation, refinement, and deodorization of lipids or essential oils containing sample matrices at an industrial scale (Ferdosh et al., 2009). By using carbon dioxide in its supercritical state as a solvent, the method allows for extracting essential oils without leaving solvent residues, ensuring purer and safer extracts for various applications (Ahmadi, 2024). Followed by the separation between CO_2 fluids and extracted materials, CO_2 gas is returned, flowing into a low-pressure CO_2 tube to be reused (Argun, 2022). Supercritical CO_2 has been widely used due to its mild conditions, non-toxicity, and wide sources.

Supercritical fluid extraction using carbon dioxide (CO_2) has gained significant attention due to its numerous advantages. SC-CO_2 extraction offers benefits such as the ease of separating the solvent after extraction, leaving minimal residue in the processed material, its non-toxic and environmentally friendly nature, and its ability to operate at low temperatures, reducing thermal degradation of the extracted products (Jian-zhong & JianYing, 2014). This method is particularly attractive as CO_2 is a non-toxic solvent, recoverable at the end of the extraction process, and can be obtained as a byproduct of various industrial processes (Abdelfattah, 2023).

Supercritical fluid extraction (SFE) with CO_2 has shown promise in various applications, including the extraction of oils from different sources. This technology leverages the enhanced solvent power of supercritical fluids, allowing for the extraction of sensitive compounds under mild conditions, which is crucial for maintaining the stability of natural products (Ren & Zhu, 2011). Additionally, SC-CO_2 extraction ensures high precision and purity in extracting essential oils from aromatic, medicinal, and pharmaceutical plants (Ahmadi, 2024).

One of the key advantages of supercritical CO_2 extraction is its ability to extract thermally labile compounds at low temperatures, producing solvent residue-free extracts. This method is useful for extracting bioactive compounds from plant matrices, offering a green and efficient extraction technique (Vági et al., 2004). Furthermore, SC- CO_2 extraction has been highlighted for its practical advantages, such as being non-toxic, non-flammable, environmentally safe, cost-effective, and suitable for extracting natural compounds (Luksta, 2023).

In summary, supercritical fluid extraction with CO_2 presents a versatile and environmentally friendly approach for extracting a wide range of compounds from various sources. Its ability to operate at low temperatures, maintain product stability, and produce high-purity extracts makes it a valuable technique in industries such as food, pharmaceuticals, and cosmetics.

MATERIAL AND METHOD

The plant was collected between May and July 2023 in four distinct regions across Albania Elbasani, Librazhdi, Pogradeci, Korca).

Figure 1. Location of *Achillea millefollium* in Elbasani, Librazhdi, Pogradeci and Korca

The process begins with the air-drying of *Achillea millefolium* specimens to preserve their morphological and chemical properties. The integrity and health of the plant are carefully monitored using a humidity monitor and microscopic examination. The essential oils from the plant are extracted using a supercritical CO_2 extraction technique. The selection and milling of plants occur in a controlled, dry, and well-ventilated atmosphere. Plant integrity and health are assessed using precise humidity sensing and microscopic analysis. The plant's particle size is carefully controlled at 0.3 mm, and a maximum humidity level of 6% is maintained according to the standards of supercritical fluid extraction (SCFE) technology. Extraction of solids from ground materials, pellets, or granules is consistently carried out using food-grade carbon dioxide as a solvent. The equipment involved in the extraction consists of two separators for removing CO_2 from the extract before recycling, as well as two or more extractors operating above the CO_2 critical pressure. The extraction process involves raising the temperature and pressure of the carbon dioxide gas until it reaches the supercritical state.

This is facilitated by a heater and a high-pressure pump. The extractors contain organic raw material through which the supercritical CO_2 passes. After obtaining the entire CO_2 extract, it is diluted in n-hexane at a ratio of 1:2. The chemical composition of *Achillea millefolium* is then determined using Gas Chromatography with Flame Ionization Detector (GC-FID). This comprehensive method allows for precise analysis of the extracted essential oils.

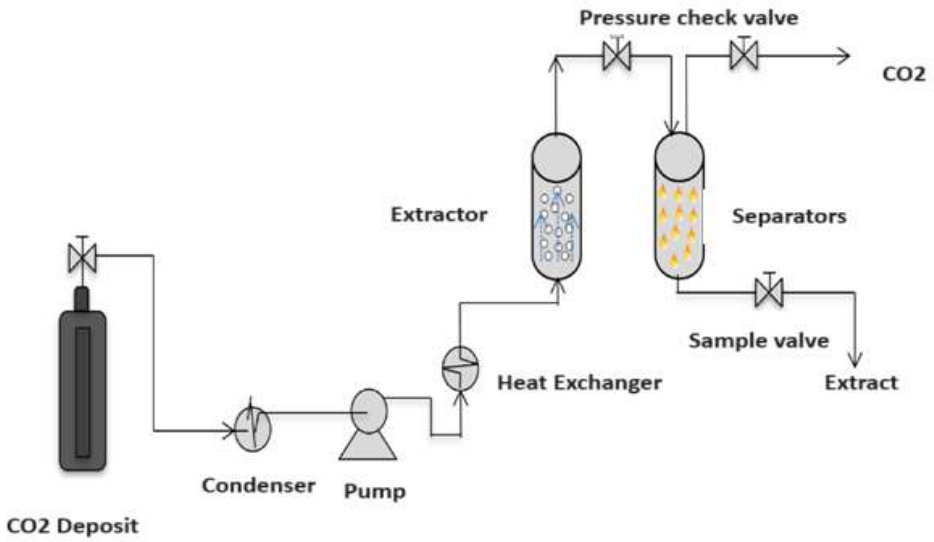


Figure 2. Scheme of CO_2 extractor

RESULTS AND DISCUSSION

Essential oil of *Achillea millefolium* samples from Pogradeci, Elbasani, Korca and Librazhdi were analyzed using GC/FID technique. According to GC- FID the results for chemical composition of Achillea millefolium are shown below for each region in percentage. Main chemical components are shown in the Table 1.

The main component of Achillea millefolium are the sequiterpenes: **β-Caryophyllene** from 19.8 % in Pogradeci to 24 % in Korca. **β-Caryophyllene** with anti-inflammatory, antimicrobial, antibacterial, and antioxidant properties (Scandiffio R, Geddo 2020). The second most abundant component is **Germancrene** from 11% in Librazhdi – 12.4 % in Korca. Germancrene has antibacterial and antifungal activities (Badalamenti, Natale 2022). **Cedrene** from 2,9 % in Librazhd and Pogradec – 4,31 % in Elbasan. Cedrene possesses antimicrobial properties, which can be beneficial in food preservation and in the development of natural antimicrobial agents (Yang, 2023).

Table 1. Percentage of chemical composition of *Achillea millefollium*

Chemical composition	**Elbasani (%)**	**Librazhdi (%)**	**Korca (%0**	**Pogradeci (%)**
a-Thujone	1.43	2.5	2.2	3
a-Pinene	2.21	2.8	1.6	2.2
Sabinene	6.7	6.4	4	3.6
1.8-Cineole	8.08	7	7.4	6.2
a-Phellandrene	1.39	2.1	1.2	4
p-Cymene	4.2	5.6	5.2	4.6
a-Terpinene	2.56	2.2	1.2	3.1
Terpinolene	1.26	1.9	1.4	2
Pinocarvone	1.84	2	1.2	1.9
Camphor	2.52	2.3	2.4	2
Linalol	2.9	3.2	2.4	1.9
Borneol	1.71	2.2	3.2	3.4
Geraniol	3.31	3.4	3.8	4
Dodecanol	1.69	3	3.4	3.2
Bornyl acetate	5.19	4.8	6	5.6
b-Caryophyllene	21.58	20	24	19.8
b-Pinene	7.1	6.4	6.2	6.4
Longipinene	1.95	2.1	2.1	2.1
Germancrene	11.3	11	12.4	11.9
Cedrene	4.31	2.9	3.2	2.9
Humulene	2.37	2.1	2.1	2.1
Cadinene	2.66	2.9	2.5	2.9
Caryophyllene oxide	1.72	1.2	0.9	1.2

The other chemical components of *A. millefolium* are the monoterpenes: **1,8 Cineole** 6.2% in Pogradec-8.08 % in Elbasani; 1.8 Cineole (eucalyptol) is a monoterpenoid that has many pharmacological effects, including anti-inflammatory and antioxidant properties. It has been used to treat respiratory and cardiovascular diseases and is clinically applied in rhinosinusitis, COPD, asthma, and bronchitis. (Cosima C. Hoch 2023). **β- pinene** is 6.2 % in Korce to the highest in Elbasan 7.1 %. A wide range of pharmacological activities have been reported, including antibiotic resistance modulation, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, anti-Leishmania, and analgesic effects (Salehi B, Upadhyay 2019). Geraniol 3.31% in Elbasani and 4% in Pogradeci. **Geraniol** has antioxidant and anti-inflammatory properties. In vitro and in vivo studies have shown the activity of geraniol against prostate, bowel, liver, kidney and skin cancer (Mączka W 2020). **Sabinene** (3.6 % in Pogradeci 6.7 % in Elbasani). The compound has been shown to exhibit anti-inflammatory properties (Kim, 2024; An et al., 2013). The antimicrobial properties of sabinene have also been documented, with studies showing its effectiveness against various bacterial strains, including **Streptococcus mutans*, where it inhibits growth and biofilm formation (Park et al., 2019). **Borneol** 1.71 % in Elbasani and 3.4 % in Pogradeci. Borneol has significant anti-inflammatory and analgesic effects. Research indicates that borneol can reduce nociceptive behavior and inflammatory responses in animal models, suggesting its potential as an analgesic agent (Almeida et al., 2013; Bansod et al., 2020). **Bornyl acetate** from 4.6 % in Librazhdi to 6% in Korca.

It is recognized for its diverse biological activities and potential applications in various fields, including medicine and agriculture. Bornyl acetate is a multifaceted compound with significant biological activities, including anti-inflammatory, antioxidant (Kim et al., 2013), and antimicrobial (Ivanova, 2023) effects. Its role as a precursor in the synthesis of camphor and its applications in cosmetics further emphasize its importance in both scientific research and practical applications. **Cymene** 4.2 % in Elbasani and 5.6% in Librazhdi. p-cymene exhibits notable antimicrobial properties. Research has shown that it can enhance the antimicrobial effects of other compounds, such as carvacrol and nisin, making it a valuable additive in food preservation and safety (Marchese et al., 2017).

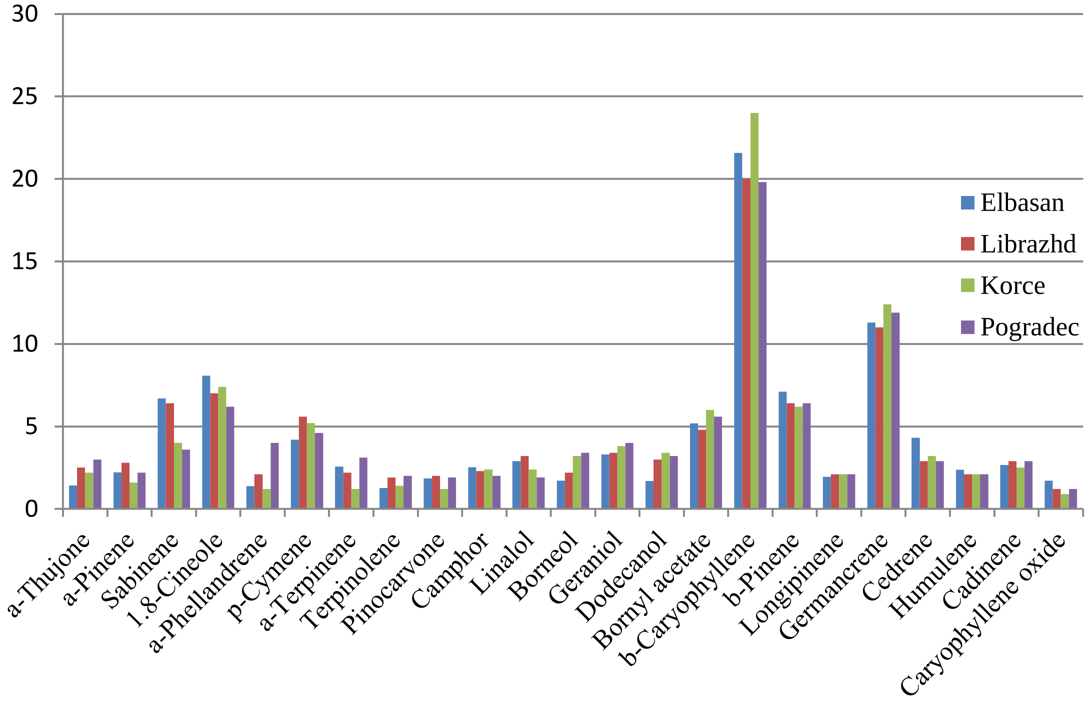


Figure 3. *Achillea millefolium* chemical composition in 4 different regions in Albania

CONCLUSIONS

Achillea millefolium, also known as yarrow, is a perennial herb from the Asteraceae family, widely found across Albania. Collected in four different Albanian regions

(Elbasani, Librazhdi, Pogradeci and Korca), bioactive compounds from *A. millefolium* are extracted using supercritical carbon dioxide (CO2) extraction (SFE- CO_2) and analyzed using GC/FID. The SFE- CO_2 method yields essential oils rich in monoterpenes such as 1.8-Cineole (6.2%-8.08%) and b-Pinene (6.2%-7.1%), as well as sesquiterpenes including Caryophyllene (19.8%-24%) and Germancrene (11%-12.4%). Variations in chemical composition percentages may be attributed to geographic and geological factors.

The SFE- CO2 extraction method has shown potential in producing high-quality extracts with enhanced therapeutic properties, offering advantages over traditional methods such as improved extraction efficiency and preservation of sensitive compounds. With its diverse biological activities and traditional medicinal uses, Achillea millefolium has garnered significant interest. Ongoing studies utilizing *A. millefolium* in nanotechnology have demonstrated promising effects, providing a practical distribution system. Further research into the composition and applications of *A. millefolium* extracts is likely to enhance their utility in medicine, agriculture, and the food industry.

REFERENCES

Abdelfattah, M. (2023). Green solvents for the extraction of mycotoxins during the analysis of food products: a mini review. Advances in Basic and Applied Sciences, 1(1), 21-32. https://doi.org/10.21608/abas.2023.210759.1021

Ahmadi, K. (2024). Evaluation of a new process for extracting essential oil from aromatic, medicinal, and pharmaceutical plants. E3s Web of Conferences, 527, 01008. https://doi.org/10.1051/e3sconf/202452701008

Al-Ezzy, Ruqaya & Anee, Rafal & Ibrahim, Niran. (2018). Assessments of Immunological Activity of Achillea Millefolium Methanolic Extract on Albino Male Mice. Journal of Pharmacy and Pharmacology. 6. 10.17265/2328-2150/2018.06.002.

Aliasghari, A., Majd, S. A., Khorasgani, M. R., Khosravi, F., Shokri, D., & Mohammadi, A. (2017). Antibacterial Effects of Camellia sinensis and Achillea millefolium on several antibiotic-resistant bacteria. *Eur J Med Plants*, *19*(4), 1-8.

Almeida, J., Souza, G., Silva, J., Saraiva, S., Oliveira-Júnior, R., Quintans, J., … & Quintans, L. (2013). Borneol, a bicyclic monoterpene alcohol, reduces nociceptive behavior and inflammatory response in mice. The Scientific World Journal, 2013(1). https://doi.org/10.1155/2013/808460.

Alzomor, Abdulkarim & Al-Absi, Nada & Al-Hssany, Abubaker & Al-Salahi, Helmy & Almushra'a, Ashgan & Zomor, Abdulkarim. (2023). Citation: Saudi Journal of Medical and Pharmaceutical Sciences Investigate the Effects of Achillea millefolium Plant Extract as A Hepatoprotection on Carbon Tetrachloride-Induced Liver Toxicity in Female Rats. 10.36348/sjmps. 2022.v08i05.00X.

An, B., Kang, J., Yang, H., Jung, E., Kang, H., Choi, I., … & Jeung, E. (2013). Anti-inflammatory effects of essential oils from chamaecyparis obtusa via the cyclooxygenase-2 pathway in rats. Molecular Medicine Reports, 8(1), 255-259. https://doi.org/10.3892/mmr.2013.1459.

Badalamenti, Natale & Bruno, Maurizio & Formisano, Carmen & Rigano, Daniela. (2022). Effect of Germacrene-Rich Essential Oil of Parentucellia latifolia (L.) Caruel Collected in Central Sicily on the Growth of Microorganisms Inhabiting Historical Textiles. Natural Product Communications. 17. 10.1177/1934578X221096963

Bansod, S., Chilvery, S., Saifi, M., Das, T., Tag, H., & Godugu, C. (2020). Borneol protects against cerulein-induced oxidative stress and inflammation in acute pancreatitis mice model. Environmental Toxicology, 36(4), 530-539. https://doi.org/10.1002/tox.23058

Cosima C. Hoch, Julie Petry, Lena Griesbaum, Tobias Weiser, Kathrin Werner, Michael Ploch, Admar Verschoor, Gabriele Multhoff, Ali Bashiri Dezfouli, Barbara Wollenberg,1,8-cineole (eucalyptol): A versatile phytochemical with therapeutic applications across multiple diseases,Biomedicine & Pharmacotherapy,Volume 167,2023, 115467,ISSN 0753-3322,https://doi.org/10.1016/j.biopha.2023.115467.

Durak, F., Aydın, A., & Sökmen, M. (2023). Optimization of supercritical fluid CO_2 extraction of Opoponax essential oil (C. erythraea). *International Journal of Secondary Metabolite*, *10*(1), 38-47.

El-Sadek, S & El-Gendy, A.A.M. & Tohamy, Mohamad & El-Aa, M. (2007). Anti-inflammatory, antipyretic and analgesic effect of Achillea millefolium and Salix plants. Journal of Veterinary Medical Research. 17. 10.21608/jvmr.2007.77898

Ferdosh, Sahena & Sarker, Md Zaidul & Jinap, Selamat & Karim, Alias & Abbas, K.A. & Norulaini, Nik & Kadir, Mohd. (2009). Application of supercritical CO_2 in lipid extraction - Areview. Journal of Food Engineering. 95. 240-253. 10.1016/j.jfoodeng.2009.06.026.

Ghobadian, Zahra & Hafezi Ahmadi, Mohammad & Rezazadeh, Leila & Hosseini, Ehsan & Kokhazadeh, Taleb & Ghavam, Samiramis. (2015). In Vitro Evaluation of Achillea Millefolium on the Production and Stimulation of Human Skin Fibroblast Cells (HFS-PI-16). Medical Archives. 69. 212. 10.5455/medarh.2015.69.212-217.

Hajisalem, Telka & Ghaffary, Saba & Nejati, Babak & Mashayekhi, Simin & Fatemeh, Fathiazad & Shokri, Javad & Bateni, Amin. (2019). Effect of Achillea millefolium Mouthwash on Oral Mucositis Induced by Chemotherapy in AML Patients. Jundishapur Journal of Natural Pharmaceutical Products. In Press. 10.5812/jjnpp.14077. https://doi.org/10.1016/j.seppur.2022.122593.

Hassanzadeh-Kiabi F, Negahdari B. Antinociceptive synergistic interaction between Achillea millefolium and Origanum vulgare L. extract encapsulated in liposome in rat. Artif Cells Nanomed Biotechnol. 2018 Aug; 46(5): 994-1000. doi: 10.1080/21691401.2017.1354303. Epub 2017 Jul 18. PMID: 28720004

Hassanzadeh-Kiabi F, Negahdari B. Antinociceptive synergistic interaction between Achillea millefolium and Origanum vulgare L. extract encapsulated in liposome in rat. Artif Cells Nanomed Biotechnol. 2018 Aug;46(5):994-1000. doi: 10.1080/21691401.2017.1354303. Epub 2017 Jul 18. PMID: 28720004.

Ivanova, S., Pashova, S., Dyankov, S., Georgieva, Y., Ivanov, K., Benbassat, N., … & Karcheva-Bahchevanska, D. (2023). Chemical composition and future perspectives of essential oil obtained from a wild population of stachys germanica l. distributed in the balkan mountains in bulgaria. International Journal of Analytical Chemistry, 2023, 1-11. https://doi.org/10.1155/2023/4275213

Jian-zhong, J. and JianYing, T. (2014). Optimization of supercritical extraction of oil from onion by supercritical co2. Asian Journal of Chemistry, 26(1), 142-144. https://doi.org/10.14233/ajchem.2014.15353

Kaloshi, Edlira, et al. "Determination of Phytochemical Components of ACHILLEA MILLEFOLIUM in Different Areas of Albania. International Symposium for Environmental Science and Engineering Research." *ISESER2023*, 18 Sept. 2023, Konya, Turkey, iseser.com, pp. 259.

Khan AU, Gilani AH. Blood pressure lowering, cardiovascular inhibitory and bronchodilatory actions of Achillea millefolium. Phytother Res. 2011 Apr;25(4):577-83. doi: 10.1002/ptr.3303. Epub 2010 Sep 20. PMID: 20857434.

Kim, S. (2024). Anti-inflammatory effect of chamaecyparis obtusa (siebold & zucc.) endl. leaf essential oil. Molecules, 29(5), 1117. https://doi.org/10.3390/molecules29051117.

Luksta, I. (2023). Extraction of apple pomace using supercritical co2 extraction. Environmental and Climate Technologies, 27(1), 980-988. https://doi.org/10.2478/rtuect-2023-0071

Mączka W, Wińska K, Grabarczyk M. One Hundred Faces of Geraniol. Molecules. 2020 Jul 21;25(14):3303. doi: 10.3390/molecules25143303. PMID: 32708169; PMCID: PMC7397177.

Marchese A, Barbieri R, Coppo E, Orhan IE, Daglia M, Nabavi SF, Izadi M, Abdollahi M, Nabavi SM, Ajami M. Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. Crit Rev Microbiol. 2017 Nov;43(6):668-689. doi: 10.1080/1040841X.2017.1295225. Epub 2017 Mar 27. PMID: 28346030.

Mehmet Emin Argun, Mustafa Şamil Argun, Fatma Nur Arslan, Bilgehan Nas, Havva Ates, Suheyla Tongur, Ozgur Cakmakcı,Recovery of valuable compounds from orange processing wastes using supercritical carbon dioxide extraction, Journal of Cleaner Production, Volume 375, 2022, 134-169, ISSN 0959-6526, https://doi.org/10.1016/j.jclepro.2022.134169.(https://www.sciencedirect.com/science/article/pii/S0959652622037416)

Mirzania, F., Sarrafi, Y., & Farimani, M. (2018). Comparison of chemical composition, antifungal and antibacterial activities of two populations of salvia macilenta boiss. essential oil. Records of Natural Products, 12(4), 385-390. https://doi.org/10.25135/rnp.37.17.10.166

Mothana, R. (2012). Antimicrobial and antioxidant activities and gas chromatography mass spectrometry (gc/ms) analysis of the essential oils of ajuga bracteosa wall. ex benth. and lavandula dentata l. growing wild in yemen. Journal of Medicinal Plants Research, 6(15). https://doi.org/10.5897/jmpr11.1565.

Okkay, U., Ferah Okkay, I., Cicek, B., Aydin, I. C., Ertugrul, M. S., Bayram, C., ... & Hacimuftuoglu, A. (2021). Achillea millefolium alleviates testicular damage in paclitaxel-intoxicated rats via attenuation of testicular oxido-inflammatory stress and apoptotic responses. *Andrologia*, *53*(5), e14028.

Park, B., Kim, B., Kim, K., & You, Y. (2019). Sabinene suppresses growth, biofilm formation, and adhesion of streptococcus mutans by inhibiting cariogenic virulence factors. Journal of Oral Microbiology, 11(1), 1632101. https://doi.org/10.1080/20002297.2019.1632101.

Ren, X. and Zhu, W. (2011). Optimal conditions for extraction of oil from fenugreek (trigonella foenum - graecum l.) by supercritical co₂ fluids (sfe-co₂). Advanced Materials Research, 236-238, 2980-2983. https://doi.org/10.4028/www.scientific.net/amr.236-238.2980.

Rezaei, Shahla & Ashkar, Fatemeh & Koohpeyma, Farhad & Mahmoodi, Marzieh & Gholamalizadeh, Maryam & Mazloom, Zohreh & Doaei, Saeid. (2020). Hydroalcoholic extract of Achillea millefolium improved blood glucose, liver enzymes and lipid profile compared to metformin in streptozotocin-induced diabetic rats. Lipids in Health and Disease. 19. 10.1186/s12944-020-01228-4.

Salehi B, Upadhyay S, Erdogan Orhan I, Kumar Jugran A, L D Jayaweera S, A Dias D, Sharopov F, Taheri Y, Martins N, Baghalpour N, Cho WC, Sharifi-Rad J. Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. Biomolecules. 2019 Nov 14;9(11):738. doi: 10.3390/biom9110738. PMID: 31739596; PMCID: PMC6920849.

Satari, A. H., Bhat, M. F., Hassan, R., & Masoodi, M. H. (2021). Achillea millefolium E. Mey: Phytochemistry and Pharmacological Profile. *Current Traditional Medicine*, *7*(3), 343-347.

Scandiffio R, Geddo F, Cottone E, Querio G, Antoniotti S, Gallo MP, Maffei ME, Bovolin P. Protective Effects of (*E*)-β-Caryophyllene (BCP) in Chronic Inflammation. Nutrients. 2020 Oct 26;12(11):3273. doi: 10.3390/nu12113273. PMID: 33114564; PMCID: PMC7692661.Separation and Purification Technology,Volume 306, Part A,2023,122593,ISSN 1383-5866,

Vitalini S, Beretta G, Iriti M, Orsenigo S, Basilico N, Dall'Acqua S, Iorizzi M, Fico G. Phenolic compounds from Achillea millefolium L. and their bioactivity. Acta Biochim Pol. 2011;58(2):203-9. Epub 2011 Apr 19. PMID: 21503279.

Yang, H., Wang, F., An, W., Gu, Y., Jiang, Y., Guo, H., … & Zhu, P. (2023). Comparative metabolomics and transcriptome analysis reveal the fragrance-related metabolite formation in phoebe zhennan wood. Molecules, 28(20), 7047. https://doi.org/10.3390/molecules28207047.

CYTOTOXIC ACTIVITY and WOUND HEALING EFFECT of EDIBLE MUSHROOM *Hohenbuehelia petaloides* (Bull.) Schulzer in HEALTHY 3T3 FIBROBLAST CELL LINE

Cansu KORKMAZ[1], Mehmet Emin DURU[2], Hatice GÜNEŞ[1]*

[1]Department of Biology, Faculty of Science, Muğla Sıtkı Koçman University, 48000 Muğla, Türkiye,
[2]Department of Chemistry, Faculty of Science, Muğla Sıtkı Koçman University, 48000 Muğla, Türkiye,
Corresponding author e-mail: cansukorkmaz08@gmail.com
https://orcid.org/0000-0003-3027-7687

ABSTRACT

In recent years, new therapeutic approaches that are non-toxic, lower cost and accelerate wound healing are needed. Considering the different medicinal benefits of mushrooms, mushroom extracts are gaining attention as wound healing agents. Fungi help in the wound healing process by stimulating the immune system, producing reactive oxygen species (ROS) and regulating various inflammatory intermediates. In this study, it was aimed to determine the cytotoxic activity of the edible wood fungus *Hohenbuehelia petaloides* (Bull.) Schulzer. in healthy 3T3 fibroblast cell line and its wound healing effect at a non-toxic concentration.

The collected mushroom material from Datça in Türkiye was identified and was turned into fungarium material at Muğla Sıtkı Koçman University, "Organic Chemistry and Natural Products Laboratory". *H. petalodies* was extracted (considering going from apolar to polar in the solvent system) with hot water (at about 85 °C by brewing) and the solvent of the extract was removed using a lyophilizer, respectively.

It was determined that *H. petaloides* water extract did not show toxic effect on healthy 3T3 fibroblast cell line treated for 24 and 48 hours (IC_{50}>1000 μg/mL). After 24 and 48 hours of application of *H. petaloides* water extract on healthy 3T3 fibroblast cell line, the wound area in the dose group decreased due to the increasing time compared to the control. It was determined that the wound area of 3T3 fibroblast cells treated with *H. petaloides* water extract closed at 66% and 95% in 24 and 48 hours, respectively, compared to the control group. In conclusion, in healthy 3T3 fibroblast cell line, the non-toxic dose of *H. petaloides* water extract caused 2-fold more cell migration in the wound area compared to the control at 24 and 48 hours.

Keywords: *Hohenbuehelia petaloides*, healthy 3T3 fibroblast, wound healing, cytotoxic activity

INTRODUCTION

Wounds can occur as a result of physical, chemical, thermal, microbial or immunologic events (Teymoorian et al., 2024). In recent years, there have been numerous attempts to develop new therapeutic approaches to accelerate wound healing at lower cost (Masson-Meyers et al., 2020). Chemical drugs are the common therapeutic approach to heal wounds. There are concerns about the side effects of chemical drugs because they can reach and affect non-target cells (Romano et al., 2022). And also some antiseptic solutions used in drugs, including hydrogen peroxide, chlorhexidine and povidone-iodine, have cytotoxicity effects on healthy cells and disrupt the healing process (Stan et al., 2021). Therefore, there is a need for natural products with fewer side effects and high efficacy.

Considering the different medicinal benefits of mushrooms, the use of medicinal mushroom extracts as wound healing agents is gaining popularity (Kaplan et al., 2021; Salem et al., 2022; Teymoorian et al., 2024). Studies have reported that medicinal mushrooms can help in the wound healing process by stimulating immune epithelial cells, promoting extracellular matrix formation, triggering cytokines and growth factors, producing reactive oxygen species (ROS) and regulating various inflammatory intermediates (Sharifi-Rad et al., 2020; Ge and Wang, 2023). In the study conducted by Zhao et al. (2021), the wound healing ability of different concentrations (0.02 - 0.2 μg/μL) of polysaccharides obtained from *Ganoderma amboinense* against NIH/3T3 cells at 24 and 48 hours was determined. In the study on the in-vivo and in-vitro wound healing and tissue repair effect of *Trametes versicolor* polysaccharide extract, it was determined that the wounds of treated mice, 100% and 60% of untreated mice healed within 14 days (Teymoorian et al., 2024).

In this study, it was aimed to determine the cytotoxic activity of the edible wood fungus *Hohenbuehelia petaloides* (Bull.) Schulzer. in healthy 3T3 fibroblast cell line and its wound healing effect at a non-toxic concentration.

MATERIAL AND METHOD

1. Mushroom Material

Hohenbuehelia petaloides (Bull.) Schulzer was freshly collected from mouse ear grass and woody tree remains in a mixed forest of red pine, pistachio pine and palm trees in Datça Peninsula, an ecotourism area in the Aegean Region of Anatolia. The collected fresh mushroom material was identified and some of them (about 5 grams, numbered with the code CK010) ws turned into fungarium material at Muğla Sıtkı Koçman University, "Organic Chemistry and Natural Products Laboratory".

2. Mushroom Extract

The dried 300 gram of mushroom was extracted with hot water (at about 85 °C by brewing) and the solvent of the extract was removed using a lyophilizer, respectively (considering going from apo to po in the solvent system).

3. Cytotoxic Activity

Healthy 3T3 fibroblast cell line was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-dipenyltetrazolium bromide) assay (Plumb.,1997). Cells were plated in 96-well plates in triplicate with a density of 1000 cells/well. The cells were treated with water extract of *H. petaloides* (0-2000 µg/ mL) after culturing for 24 h followed by incubation for 24 and 48 h. Control group cells were left untreated. 3T3 fibroblast cell line was incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. IC_{50} value was determined using the AAT Bioquest. The data were expressed as the mean ± standard deviation (SD) of triplicate independent experiments and analyzed one sample t test using EXCEL PRO 2019-QI Macros. The percent of cytotoxicity was calculated in accordance with the following formula (Ugur et al., 2017).

% Cytotoxicty= [Control OD-Sample OD)/Control OD] x 100

4. Wound Healing Effect

90% confluent cells were passaged and $5x10^5$ cells were inoculated into 24-well cell culture dishes. The microscopic appearance of the 3T3 fibroblast cell line is given in Figure 1. Cells were incubated at 37 ºC, 5% CO2 and 98% humidity in an incubator for 20-24 hours. After incubation, a line (wound) was created in the center of each well with a 200 µl pipette tip. After the cells were washed twice with PBS, *H. petaloides* water extract was added at a concentration (500 µg/mL) at which it showed no toxic effect at 24 and 48 hours. During the incubation period, at 24 and 48 hours, the migration of cells along the gap formed by drawing a line was followed and photographed under an inverted microscope. Cell migration or wound healing was determined by observing the amount of cells migrating into the formed spaces. The wound area photographs obtained at the end of the studies were analyzed with ImageJ software. The percentage of wound healing is calculated by (C0 – C24)*100/C0, Whereas C0 = mean distance of wound at 0 h and C24 = mean distance of wound at 24h (Roy et al., 2023).

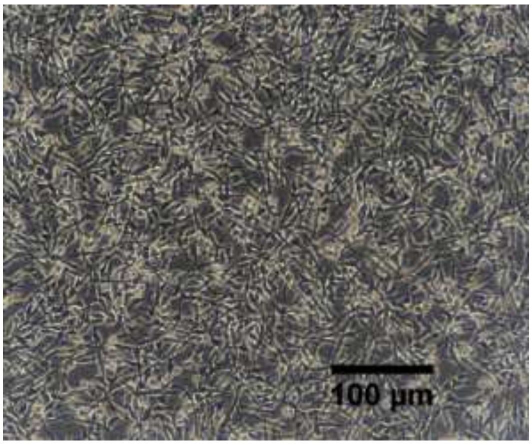


Figure 1. Microscopic view of healthy 3T3 fibroblast cell line

5. Statistical Analysis

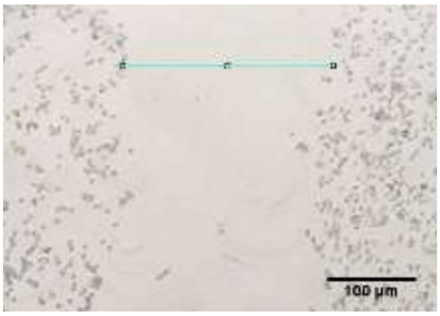
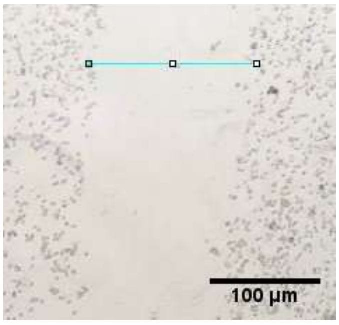
The data were expressed as the mean ± standard deviation (SD) of triplicate independent experiments and analyzed via Two-ways analysis of variance (ANOVA) followed by using EXCEL PRO 2019-QI Macros, and GraphPad Prism version 8 Software. $p < 0.0001$ was considered to be statistically significant.

RESULTS AND DISCUSSION

It was determined that *H. petaloides* water extract did not show toxic effect on healthy 3T3 fibroblast cell line treated for 24 and 48 hours (IC_{50}>1000µg/mL). In recent studies on the wound healing effect of fungi, the antioxidant and proliferation effect of Talaromyces purpureogenus extract on cells was determined (Hu et al., 2023). In another study, the positive wound healing potential of Lignosus rhinoceros extract was determined (Yap et al., 2023).

In this study, a significant difference was detected in the wound area in the area where H. petaloides water extract (500µg/mL concentration) was applied compared to the control group ($p < 0.001$). When Table 1 is examined, after 24 and 48 hours of application of *H. petaloides* water extract in healthy 3T3 fibroblast cell line, the wound area in the dose group decreased with increasing time compared to the control. It was determined that the wound area of 3T3 fibroblast cells treated with *H. petaloides* water extract was closed by 66% and 95% at 24 and 48 hours, respectively, compared to the control group. In the control group, 34% and 45% of the wound area closed at 24 and 48 hours, respectively (Figure 2). Compared to the control group, the study was terminated at 48 hours in the area treated with *H. petaloides* water extract as it filled the wound area at 48 hours. In conclusion, *H. petaloides* water extract (500 µg /mL) at a non-toxic dose in healthy 3T3 fibroblast cell line caused 2-fold more cell migration in the wound area compared to the control at 24 and 48 hours. In this respect, it is a source of hope for future preclinical and adjuvant strategy research for fungal-based wound healing therapies that can help tissue regeneration and healing of skin wounds.

Table 1. The effect of 24 and 48 hours application of *H. petaloides* water extract on migration of healthy 3T3 fibroblast cell line

	Control	**Water extract of *H. petaloides***
0s		
24s		
48s		

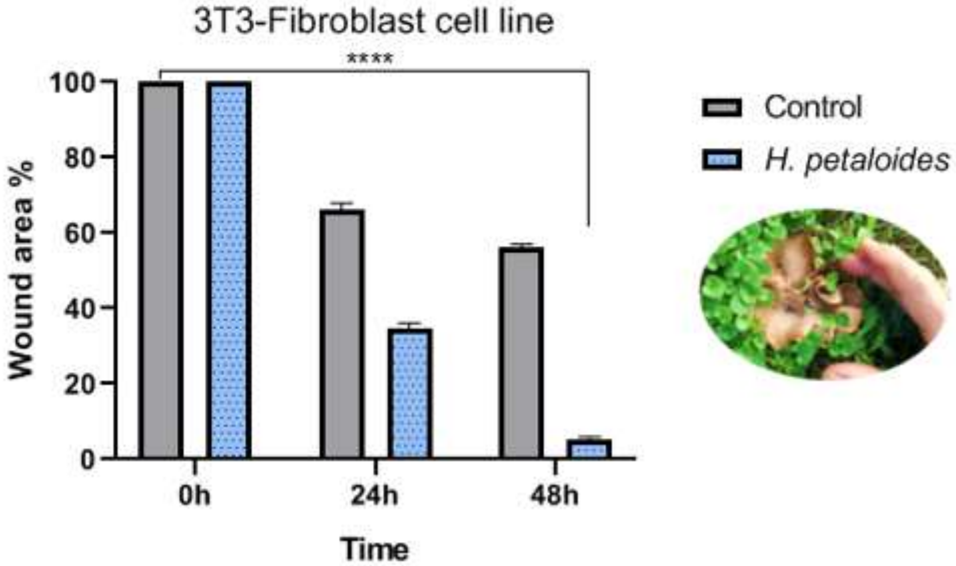


Figure 2. Percentage column graph of 24 and 48 hours application of *H. petaloides* water extract in healthy 3T3 fibroblast cell line
There was a significant difference in the wound area in the area where *H. petaloides* water extract was applied compared to the control group ($p < 0.001$).

CONCLUSIONS

H. petaloides water extract (500 µg /mL) at a non-toxic dose in healthy 3T3 fibroblast cell line caused 2-fold more cell migration in the wound area compared to the control at 24 and 48 hours. In this respect, it is a source of hope for future preclinical and adjuvant strategy research for fungal-based wound healing therapies that can help tissue regeneration and healing of skin wounds.

ACKNOWLEDGEMENT

This study was supported by Muğla Sıtkı Koçman University Scientific Research Projects Unit (BAP) with the project number 21/129/02/1/4. The authors would like to thank “YÖK 100/2000 Natural and Herbal Products/ Cosmetic” programs for providing PhD scholarship to Cansu Korkmaz. We would also like to thank TUBITAK (BIDEB 2211-A) and BIDEB 2250 projects for their support to Cansu Korkmaz.

REFERENCES

AAT Bioquest, Available from: https://www.aatbio.com/tools/ic50-calculator., 03.02.2024.

Cory, G., "Scratch-wound assay.", Methods Mol Biol., 769, 25-30, (2011).

Ge, Y. & Wang, Q. 2023. Current research on fungi in chronic wounds. Front. Mol. Biosci. 9, 1057766.

Jonkman, J.E., Cathcart, J.A., Xu, F., Bartolini, M.E., Amon, J.E., Stevens, K.M., Colarusso, P.2014. An introduction to the wound healing assay using live-cell microscopy, Cell Adh Migr., 8 (5), 440-51.

Kaplan, Ö. et al. 2021. Microwave-assisted green synthesis of silver nanoparticles using crude extracts of Boletus edulis and Coriolus versicolor: Characterization, anticancer, antimicrobial and wound healing activities. J. Drug Deliv. Sci. Technol. 64, 102641.

Masson-Meyers, D. S. et al. 2020. Experimental models and methods for cutaneous wound healing assessment. Int. J. Exp. Pathol. 101(1–2), 21–37.

Plumb, J.A. 1999. Cell sensitivity assays : the MTT assay. Methods Mol Med, 1999. 28: 25-30.

Romano, V. et al. 2022. Cell toxicity study of antiseptic solutions containing povidone-iodine and hydrogen peroxide. Diagnostics 12(8), 2021.

Roy, D., Liu, G. S., Wang, A. Z., Zhou, B., Raza, G., Merugu, S. B., ... & Zhao, B. 2023. Construction and stable gene expression of Agr2xpd1 bi-specific antibody that enhances attachment between T-cells and lung tumor cells, suppress tumor cell migration and promoting Cd8 expression in cytotoxic T-cells. Saudi Pharmaceutical Journal, 31(1), 85-95.

Salem, S. H. et al. 2022. The antimicrobial, antibiofilm, and wound healing properties of ethyl acetate crude extract of an endophytic fungus Paecilomyces sp. (AUMC 15510) in earthworm model. Sci. Rep. 12(1), 19239.

Sharifi-Rad, J. et al. 2020. Mushrooms-rich preparations on wound healing: From nutritional to medicinal attributes. Front. Pharmacol. 11, 567518.

Stan, D. et al. 2021.Wound healing applications of creams and "smart" hydrogels. Exp. Dermatol. 30(9), 1218–1232.

Teymoorian, S. K., Nouri, H., & Moghimi, H. 2024. In-vivo and in-vitro wound healing and tissue repair effect of *Trametes versicolor* polysaccharide extract. Scientific Reports, 14(1), 3796.

Ugur, D., Günes, H., Günes, F., Mammadov R. 2017. Cytotoxic Activities of Certain Medicinal Plants on Different Cancer Cell Lines. Turk. J. Pharmaceut. Sci., 2017, 222–230.

Zhao, S., Lei, M., Xu, H., He, H., Suvorov, A., Wang, J., ... & Chen, L. 2021. The normal cell proliferation and wound healing effect of polysaccharides from *Ganoderma amboinense*. Food Science and Human Wellness, 10(4), 508-513.

DISTRIBUTION OF ROOT-KNOT NEMATODE SPECIES (*MELOIDOGYNE* SPP.) IN POTATO PRODUCTION AREAS OF SOUTHWESTERN BULGARIA

Lilyana KOLEVA[1], Georgi DIMITROV[1], Radoslava DIMOVA[1]

University of Forestry, Sofia, 10 Kliment Ohridski Blvd, 1756, Sofia, Bulgaria

Corresponding author e-mail: lmarkova@ltu.bg

ABSTRACT

In Europe, potatoes (*Solanum tuberosum* L.) are regarded as one of the principal food crops, alongside wheat. The majority of potato production in Bulgaria is concentrated in the following regions: Pazardzhik, Plovdiv, Smolyan, Samokov and Blagoevgrad. It is established that in these regions, in the recent past, agricultural areas were predominantly cultivated for tobacco (*Nicotiana* sp. L.), which, along with potatoes, has a number of common pests and pathogens, including phytonematodes and particularly root-knot nematodes (*Meloidogyne* spp.). This represents a significant risk for potato growers, given the established presence of these pests in these areas and their capacity to infest a diverse range of hosts. The aim was to study the distribution of root-knot nematodes of the genus Meloidogyne in potato production areas where tobacco had been grown as a monoculture for decades.

The results presented in this study were obtained during the period 2022–2023. They are based on the monitoring of potato-producing areas, with a particular focus on 12 monitoring points occupied by potatoes. These points were selected due to the presence of preliminary information on root-knot nematode problems. The findings of the research indicate that root-knot nematodes are widespread in the potato fields in southwestern Bulgaria. A total of nine different genera of plant-parasitic nematodes were identified in the soil samples. The genera *Meloidogyne*, *Globodera*, *Pratylenchus*, *Paratylenchus*, *Tylenchorynchus*, *Tylenchus*, *Rotylenchus*, *Helicotylenchus* and *Hemicycliophora*, and those belonging to the Meloidogyne genus, were identified in 75% of the sampled fields. Potato tubers infected with nematodes of the genus Meloidogyne were found in 90% of the examined samples.

The information obtained is important to predict the spread of the infection and the preparation of an effective nematode management strategy.

Keywords: root-knot nematode, potato, tobacco

INTRODUCTION

The potato (*Solanum tuberosum* L.) is considered one of the principal food crops in Europe, along with wheat. The majority of potato production in Bulgaria is concentrated in the regions of Pazardzhik, Plovdiv, Smolyan, Samokov and Blagoevgrad. Semi-mountainous areas above 800-900 m and up to 1000-1500 m above sea level are considered suitable for growing seed potatoes, while areas at lower altitudes and on plains are suitable for growing early and mid-early varieties for consumption. It is known that, in the recent past, agricultural land in these areas was mainly occupied by tobacco (*Nicotiana* sp. L.), which shares with potatoes common pests, including plant parasitic nematodes and in particular root-knot nematodes (*Meloidogyne* spp.). This represents a significant risk to potato production given the established presence of these pests in the region and their documented ability to infest a wide range of host plants (Samaliev, 1990; Etropolski et al., 1994). Root-knot nematodes (*Meloidogyne* spp.) represent a significant economic threat to agriculture, with the potential to inflict substantial damage to potato crops (*Solanum tuberosum* L.) across a range of climatic zones, including temperate latitudes and tropical and subtropical regions (Bairwa et al., 2022; Abrantes et al., 2023). In the regions of Bulgaria where potatoes are cultivated for human consumption, the threat posed by root-knot nematodes remains underestimated. Notwithstanding the fact that considerable damage has been identified in certain potato-cultivating regions and the prevalence of root-knot nematodes is on the rise (Samaliev and Baicheva, 2010; Samaliev and Kalinova, 2013). It is important to note that certain species of the root-knot nematodes on potato, namely *Meloidogyne chitwoodi*, *M. enterolobii* and *M. fallax*, are currently classified as quarantine pests. No evidence exists to suggest that these species have been established in Bulgaria. Infected fields may be subject to direct and indirect damage caused by root-knot nematodes, which can have a detrimental impact on the potato crop. In addition to the considerable losses in the yield, the inferior quality of the resulting production limits the potential for its commercialisation. The decline in revenue is becoming increasingly pronounced, both within the domestic market and in international markets (Bairwa et al., 2022). The practice of monoculture farming provides a permanent habitat for these nematodes, resulting in the accumulation of *Meloidogyne* species over time and the establishment of a constant source of infection. In order to minimise the extent of losses incurred, it is imperative that farmers implement control measures.

The determination of the phytosanitary status of agrocenoses through monitoring represents a fundamental condition for the implementation of efficacious plant protection activities. The examination of soil and tubers to ascertain the extent of root-knot nematode infestation represents a crucial phase of the integrated plant protection system employed in Bulgarian potato cultivation.

The aim of the present study was to examine the distribution of root-knot nematodes belonging to the genus *Meloidogyne* in potato production areas where tobacco had been cultivated as a monoculture for decades.

MATERIALS AND METHODS

The findings of the present study were derived from the monitoring of areas dedicated to the cultivation of potatoes intended for human consumption and processing. The principal rationale for the selection of these areas was the cultivation of potatoes on agricultural land that had previously been used for tobacco production as a monoculture. A total of 12 monitoring points were selected in the following regions of southwestern Bulgaria: Sofia, Pernik, Kyustendil, Samokov, Pazardzhik, Blagoevgrad and Gotse Delchev during the period 2022-2023.

The samples were collected from the monitoring points at the conclusion of the vegetation period and/or at the time of harvest. The soil and tubers were subjected to analysis. The sampling was conducted in a specific sequence and pattern to ensure the consistency and comparability of the data across different fields. Soil samples were taken using a manual soil probe with a blade length of 20-30 cm and a diameter of 20-25 mm. In order to guarantee the representativeness of the aggregate sample, the number of samples taken was calculated in accordance with the dimensions of the area under investigation. The collected samples were placed in plastic bags and labelled in a clear and systematic manner. The tuber samples were collected concurrently with the soil samples and were placed in the same bag. Following the collection of the samples, they were placed in refrigerated containers. Prior to processing, the samples were stored in a refrigerator at 4°C for a maximum of two weeks. The subsequent phase of soil sample preparation for nematode extraction involved determining an average sample volume of 100 cm^3. The motile stages of the nematodes were extracted using two methods: the Cobb method and the modified Baermann funnel method (Tintori et al., 2022; Greco and Crozzoli, 2024). Following the extraction process, the samples were stored at a refrigerated temperature for a maximum of 10 days prior to the quantification and qualitative assay of the nematodes. The nematode fluid was diluted with water to 100 ml. This ensured that the total number of isolated individuals was accounted for. The resulting suspension was stirred with a magnetic stirrer and subsequently transferred to a modified Bogorov'scounting chamber. At 40× magnification under a stereomicroscope, the initial enumeration of nematodes is conducted. The plant parasitic nematodes were then excreted by a re-counting procedure. The mean number of nematodes that were extracted was determined according to the method of Peters (2013).

The available tuber material was investigated for the presence of nematode galls using a magnifying glass. The formations were separated using an entomological needle and then transferred with great care to a glass slide, where they were examined under a stereomicroscope. In addition to the aforementioned extraction methods, the detection of root-knot nematodes in plant tissues was also conducted through the use of staining techniques. The root-knot nematodes were identified morphologically in accordance with the identification keys of Jepson (1987), Karssen (2002), Perry et al. (2009) and other relevant sources.

RESULTS AND DISCUSSION

The results presented here were obtained from 12 monitoring points where tobacco was cultivated until 2010, during which time root-knot nematodes were reported (Samaliev et al., 1994).

Soil characteristics

The study areas were distinguished by a variety of soil types. Soil analyses showed that all samples had low humus content (Table 1). The soils were predominantly sandy. The lowest humus content was observed in the soil samples collected in the area of G. Delchev (0.7%), while the highest was noted in the samples from Dorkovo (2.6%). The soil samples collected in the Yundola and Velingrad region exhibited low pH values (5.1–5.9), whereas the pH of the soil samples from the Hadzhidimovo and Ognyanovo regions (6.3 and 6.9, respectively) and Samokov (Samokov and Alino) ranged from neutral to slightly alkaline (7.0–7.5).

Table 1. Soil characteristics of monitoring points in southwestern Bulgaria

	Monitoring points /Location	**crop**	**Soil type**	**Humus content, % 0–30cm**	**pH (KCl)**
1.	Gotse Delchev 41.569064, 23.760338	Potato	Eroded Choromic Cambisols	0.7	5.6
2.	Banichan 41.620586, 23.742062	Potato	Choromic Cambisols	0.95	5.1
3.	Ognyanovo 41.608738, 23.778636	Potato	Fluvisols/ Mollic Fluvisols	1.73	6.9
4.	Hadzhidimovo 41.520661, 23.859279	Potato	Eroded Choromic Cambisols	1,21	6.3
5.	Bansko 41.847361, 23.465952	Potato	Eroded Choromic Cambisols	1.2	5.9
6.	Rila 42.116994, 23.118583	Tobacco	Choromic Cambisols	1.6	6.2
7.	Velingrad 42.020671, 24.004515	Potato	Albic Cambisols	2.1	5.9
8.	Kostandovo 42.020043, 24.114994	Potato	Choromic Cambisols	1.47	6.5
9.	Dorkovo 42.034816, 24.122245	Potato	Choromic Cambisols	2.6	6.5
10.	Yundola 42.066602, 23.840069	Potato	Albic Cambisols/ Umbrosols	1.9	5.1
11.	Samokov 42.331305, 23.533564	Potato	Cambisols	2.1	7.0
12.	Alino 42.380527, 23.397953	Potato	Choromic Cambisols	1.5	7.5

Plant-parasitic nematodes in soil samples

The plant-parasitic nematodes detected in soil samples from the monitoring points were identified as belonging to nine different genera. The identified genera included *Meloidogyne*, *Globodera*, *Pratylenchus*, *Paratylenchus*, *Tylenchorynchus*, *Tylenchus*, *Rotylenchus*, *Helicotylenchus* and *Hemicycliophora*. It is notable that nematodes of the genus Meloidogyne were present at 90% of the monitoring points, while those of the genus Pratylenchus were identified at 50% of the same locations. Additionally, cyst-forming nematodes belonging to the genus *Globodera* were identified. (Table 2). The genus *Meloidogyne* was not identified in the samples from the Yundola, Bansko and Kostandovo locations.

Table 2. Plant parasitic nematodes in soil samples

	Monitoring points /Location	Crop	Established genera of plant-parasitic nematodes
1.	Gotse Delchev 41.569064, 23.760338	potato	*Meloidogyne*, *Globodera*
2.	Banichan 41.620586, 23.742062	potato	*Meloidogyne*, *Pratylenchus*
3.	Ognyanovo 41.608738, 23.778636	potato	*Meloidogyne*, *Pratylenchus*, *Paratylenchus*, *Hemicycliophora*
4.	Hadzhidimovo 41.520661, 23.859279	potato	*Meloidogyne*, *Globodera*, *Pratylenchus*, *Helicotylenchus*
5.	Bansko 41.847361, 23.465952	potato	*Tylenchorynchus*, *Tylenchus*, *Rotylenchus*
6.	Rila 42.116994, 23.118583	tobacco	*Meloidogyne*, *Rotylenchus*, *Tylenchorynchus*, *Tylenchus*
7.	Velingrad 42.020671, 24.004515	potato	*Meloidogyne*, *Globodera*
8.	Kostandovo 42.020043, 24.114994	potato	*Globodera*, *Tylenchus*, *Pratylenchus*
9.	Dorkovo 42.034816, 24.122245	potato	*Meloidogyne*, *Paratylenchus*, *Helicotylenchus*
10.	Yundola 42.066602, 23.840069	potato	*Rotylenchus*, *Hemicycliophora*
11.	Samokov 42.331305, 23.533564	potato	*Meloidogyne*, *Pratylenchus*
12.	Alino 42.380527, 23.397953	potato	*Meloidogyne*, *Pratylenchus*, *Paratylenchus*

Quantitative analysis of the motile stages of the genus Meloidogyne in soil samples

The number of motile juvenile stages (J2) and males of the genus Meloidogyne in the 9 monitoring points where their presence was identified ranged from 6 to 171.3 nematodes per 100 cm³ of soil. It should be noted that the number of nematodes in each sample, collected within the same area, varied considerably (Table 3). For instance, the density of nematodes in the samples from Gotse Delchev exceeded 150 per 100 cm³ of soil, reaching a maximum of 171.3 per 100 cm³. In contrast, the number of nematodes in the other monitoring points from the area was below 50 per 100 cm³. The density of nematodes in the Banichan samples was 23.1 per 100 cm3 soil, while in the Hadzhidimovo and Ognyanovo samples it was 21.3 per 100 cm3 soil and 39,7 nematodes/100 cm3 soil, respectively. The number of nematodes per 100 cm³ of soil was found to be below 100 in all other monitoring points. The lowest number of motile stages of root-knot nematodes was observed in the samples from Rila, with a count of 6.1 nematodes per 100 cm³ of soil.

Table 3. Presence of root-knot nematodes of the genus Meloidogyne in soil and potato tubers samples

	Monitoring points /Location	Crop /Cultivar	Nematodes ind./100 cm3 soil	Infected tubers, %, n=200
1.	Gotse Delchev 41.569064, 23.760338	Potato/Jelly	171.3	95.3
2.	Banichan 41.620586, 23.742062	Potato/Agata	23.1	19.1
3.	Ognyanovo 41.608738, 23.778636	Potato/Argos	39.7	29.6
4.	Hadzhidimovo 41.520661, 23.859279	Potato/Soraya	21.3	0
5.	Bansko 41.847361, 23.465952	Potato/Soraya	0	5.7
6.	Rila 42.116994, 23.118583	Tobacco/Rila 82	6.1	–
7.	Velingrad 42.020671, 24.004515	Potato/Jelly	95.3	89.1
8.	Kostandovo 42.020043, 24.114994	Potato/Jelly	0	12.3
9.	Dorkovo 42.034816, 24.122245	Potato/Soraya	61.6	16.6
10.	Yundola 42.066602, 23.840069	Potato/Agata	0	0
11.	Samokov 42.331305, 23.533564	Potato/Soraya	67.3	11.1
12.	Alino 42.380527, 23.397953	Potato/Soraya	59.1	7.3

Quantitative analysis of root-knot nematodes of the genus Meloidogyne isolated from potato tubers

Potato tubers infected with *Meloidogyne* sp. had malformations on the outer surface of the tuber caused by galls formed as a result of the vital activity of female nematodes. The female nematodes and egg masses were discovered within the fleshy part of the infected potato tubers. The investigation revealed that infected potato tubers were present in 90% of the samples. The percentage of infected tubers ranged from 0% in Yundola and

Hadzhidimovo to 95.3% in Gotse Delchev (Table 3). Notwithstanding the absence of root-knot nematodes in the soil samples from monitoring points Bansko and Kostandovo, the prevalence of infected tubers in these locations was 5.7 % and 12.3%, respectively. It is also noteworthy that, despite the presence of root-knot nematodes in the field in Hadzhidimovo, no infected tubers were identified. Infected potato tubers were identified in varios varieties: Argos, Soraya, Jelly and Agata, which are predominantly cultivated in this region of Bulgaria (Table 3). The Jelly variety had the highest infection rate (89.1-95.3 %). However, it was not possible to infer varietal susceptibility or resistance.

The frequency of infection with *Meloidogyne* spp. may have been influenced by a number of factors, including microclimatic conditions at the research points, varietal characteristics and the cultural and plant protection measures applied. The high percentage of fields and tubers infected with root-knot nematodes in the study areas can be explained not only by the long-term cultivation of host plants (tobacco, potatoes) in these areas, but also by local agricultural practices, where potatoes are grown in small fields, often using their own planting material. The transfer of nematodes from one field to another is facilitated by surface water and wind. Another contributing factor is the frequent absence of crop rotation and potato monoculture. Additionally, the climatic conditions and soil types prevalent in these regions of Bulgaria are conducive to the proliferation of nematodes belonging to the genus *Meloidogyne*. Furthermore, an examination of weed vegetation was conducted in the Gotse Delchev area. The majority of the collected weeds, including field mustard (*Sinapis arvensis* L.) and potato weed (*Galinsoga parviflora*), which have been documented to host root-knot nematodes (Suri and Jayasinghe, 2003, 2014), exhibited the presence of galls. The absence of root-knot nematode infestation in the Yundola region can also be attributed to the influence of temperature, soil type and altitude. The samples were obtained from an altitude of 1400 metres or above, which also corresponds to lower temperatures than those observed at other points. The authors Mutala'liah et al. (2019) observed the infection of Meloidogyne spp in areas situated at approximately 1300 m above sea level. However, no infestation was identified in areas above 1300 m above sea level, which is also related to the optimal temperature for the development of Meloidogyne spp. It is established that Meloidogyne spp. are unable to undergo complete development at low temperatures. The optimal temperature for the development of *M. javanica* and *M. arenaria* is 28 °C (Perry et al., 2009). At lower temperatures, *M. javanica* was unable to reproduce eggs (Bridge and Starr, 2007). *M. incognita* prefers a range of temperature between 25 and 30° C. The optimal temperature for hatching *M. incognita* eggs was reported to be between 15 and 30°C by Fu et al. (2012). The optimal temperature range for the penetration and development of *M. hapla* is 20–25 °C. A mean temperature of 28 °C may impede its development (Rusinque et al., 2022). In a study conducted by Asif et al. (2015), the impact of soil physicochemical attributes on the dispersion and infestation of two nematode species, namely *M. javanica* and *M. incognita*, was examined. The researchers found that physicochemical parameters, including soil temperature, soil pH, silt and clay, exerted a considerable influence on the nematode population. A negative linear correlation was observed in clay soils, whereas a positive correlation was evident in sandy loam soils with respect to root-knot nematode density.

In order to assess the risk posed by *Meloidogyne* species to potato crops, further research is needed on the species composition of root-knot nematodes in the potato-producing regions of southwestern Bulgaria. The results will contribute to a better understanding of the species composition and facilitate the development of strategies to mitigate the adverse effects. The present study revealed the presence of root-knot nematodes in association with cyst-forming nematodes in 3 of the investigated fields. It is possible that global climate change may result in the replacement of cyst nematodes by root-knot nematodes, which are better adapted to higher temperatures (da Conceição et al., 2009). The data yielded is of considerable importance in forecasting the dissemination of the infection and formulating an efficacious nematode management strategy.

CONCLUSIONS

The findings of the study indicate that root-knot nematodes are prevalent in potato crops cultivated in southwestern Bulgaria. Given the region's prominence in tobacco production, it is plausible that these nematodes have been present in these fields for an extended period.

A variety of methods are employed by farmers to control nematodes, including the use of chemicals, yet success is often limited. The disruption of the nematode life cycle through crop rotation and intercropping represents a significant challenge in this region. As a long-term strategy for the control of potato root-knot nematodes, the screening of new varieties imported from other countries or produced by the local breeding programme for resistance to Meloidogyne species should be initiated.

REFERENCES

Abrantes, I., M. T. Almeida, I. L. Conceição, I. Esteves, C. Maleita. 2023. Nematodes of potato and their management. In Potato Production Worldwide. Academic Press. 213-240.

Asif M., B. Rehman, K. Parihar, M. A. Ganai, M. A. Siddiqui. 2015. Effect of various physic-chemical factors on the incidence of root knot nematode *Meloidogyne* spp. infesting tomato in District Aligarh (Uttar Pradesh) India. Journal of Plant Sciences, 10: 234-243.

Bairwa, A., E. P. Venkatasalam, P. H. Mhatre, A. Bhatnagar, A. K. Sharma, Dalamu, ... & S. Sharma. 2022. Biology and management of nematodes in Potato. In Sustainable management of potato pests and diseases Singapore: Springer Singapore. 281-307.

Bridge, J., J. L. Starr. 2007. Plant nematodes of agricultural importance: a color handbook. Elsevier. 152.

da Conceição, I. L., M. J. da Cunha, G. Feio, M. Correia, M. C. V. dos Santos, I. M. D O. Abrantes, & M. S. D. A. Santos. 2009. Root-knot nematodes, *Meloidogyne* spp., on potato in Portugal. Nematology. 11(2): 311-313.

Etropolski, H., B. Choleva, S. Maneva. 1994. Two tobacco-producing regions. Annuaire de l'Université de Sofia, Faculté de biologie, Livre 1 – Zoologie, 85: 85-97.

Fu, Z. H., C. Du, J. X. Wu. 2012. Effects of temperature, humidity and acidity-alkalinity on growth and development of *Meloidogyne incognita*. North Hortic. 6: 137-140.

Greco, N., R. Crozzoli. 2024. Sampling and Extraction of Plant Parasitic Nematodes. In: Molinari, S. (eds) Plant-Nematode Interactions. Methods in Molecular Biology, vol 2756. Humana, New York, NY. Online ISBN 978-1-0716-3638-1, https://doi.org/10.1007/978-1-0716-3638-1_1

Jepson, S. B. 1987. Identification of root-knot nematodes (*Meloidogyne* species)., CAB International. 265

Kandouh, B. H., Hasan, A. E., Al-Hakeem, A. M., & Aridh, Z. H. (2019). A complete safe and cost-effective method for staining root-knot nematodes *Meloidogyne* spp. Plant Archives. 19, 2: 3885-3888.

Karssen, G. 2002. The plant parasitic nematode genus *Meloidogyne* Göldi, 1892 (Tylenchida) in Europe. Brill. 157

Mutala'liah, M. L., S. Indarti, A. Wibowo. 2019. The prevalence and species of root-knot nematode which infect on potato seed in Central Java, Indonesia. Biodiversitas Journal of Biological Diversity, 20(1): 11-16.

Perry, R. N., M. Moens, J. L Starr. 2009. Root-knot nematodes. CABI. 488.

Peters, A. 2013. Application and commercialization of nematodes. Applied Microbiology and Biotechnology. 97(14): 6181- 6188.

Rusinque, L., F. Nóbrega, C. Serra, M. L. Inácio. 2022. The Northern root-knot nematode: new host records in Portugal. Biology, 11(11): 1567.

Samaliev, H. 1990. Root-knot nematodes of the genus *Meloidogyne* Goeldi on tobacco, biology, ecology and opportunities for control. Dissertation, ISR-SA.

Samaliev, H., O. Baicheva. 2010. Distribution of root-knot nematodes (genus *Meloidogyne* Goeldi) on the potatoes in the Plovdiv, Troyan and Samokov regions in Bulgaria. In The Materials of the International Conference (November 30-December 3, 2010, Moscow). 458-465.

Samaliev, H., S. Kalinova. 2013. Host suitability of twelve common weeds to *Pratylenchus penetrans* and *Meloidogyne hapla* in potato fields of Bulgaria. Bulgarian Journal of Agricultural Science. 19(2): 202- 208.

Samaliev, H., S. Maneva, A. Samalieva. 1994. Population density on the yield from oriental tobacco. Annuaire de l'Université de Sofia" St. Kliment Ohridski", Faculté de biologie: Livre 1 – Zoologie, 85: 179.

Suri F., U. Jayasinghe. 2014. A survey of potato fields for root-knot nematode in Ngablak, central Java. www.eseap.cipotato.org.

Suri, F., U. Jayasinghe. 2003. A survey of potato fields for root-knot nematode in Ngablak Center Java.

Tintori, S. C., S. A. Sloat, M. V. Rockman. 2022. Rapid isolation of wild nematodes by Baermann funnel. JoVE (Journal of Visualized Experiments), (179), e63287.

ACKNOWLEDGEMENTS

This study was conducted within the project NIS-B-1210 “Identification of alternative measures for the management of root-knot nematodes on solanaceous vegetable crops to reduce the use of chemical pesticides” funded by the Scientific Sector of the University of Forestry

PRODUCTIVITY AND NUTRITIONAL VALUE OF DOMINANT SPECIES IN NATURAL PASTURES BASED ON HEDYSARUM FLEXUOSUM (SULLA) IN THE CENTRAL REGION OF ALGERIA

Hanane RAMI[1], Chafia CHIBANI[2], et Rabeha CHABACA[1]
[1] ENSA, Alger ;

[2] Laboratoire de biologie et physiologie animale (LBPA), ENS-Kouba
Corresponding author e-mail: chafia.chibani@g.ens-kouba.dz

ABSTRACT

The Mediterranean climate that characterizes the northern region of the country allows for the establishment of the most verdant natural pastures in the country. These pastures are characterized by their floristic diversity, primarily composed of grasses and legumes, serving as feed for ruminant herbivores. However, these pastures face several challenges, such as overgrazing, desertification, and climate change. The preservation and sustainable management of natural pastures have become major issues to ensure the longevity of these valuable ecosystems.
Giving pastures a significant role again could help reduce the chronic forage deficit that Algeria has been experiencing for decades. One of the solutions would be to study these pastures and their forage potential to improve them and integrate them into livestock feed. The objective of this work is to evaluate, on one hand, the floristic composition and biomass produced in the spring of a pasture in the Dellys region (Wilaya of Boumerdes), and on the other hand, to determine the chemical composition and "*in vitro*" digestibility of organic matter (OMD) of the dominant species in the pasture. This study reveals that spring productivity is 5.31 tons, which remains variable from year to year. The floristic study shows an abundance of legumes with an abundance rate of 50%, where *Hedysarum flexuosum* (sulla) is the dominant species with a total abundance of 28.6%. Grasses represent 35.7%, and other species note an abundance of 14.2%. The results of the chemical composition show a highly significant difference between species, particularly for crude protein (CP) content (8 to 20%). *Hedysarum flexuosum* presents the highest value for legumes (20.5%), and Allopecurus pratensis (11.8%) for grasses. The *in vitro* measured digestibility of OM shows average to good levels, between 60 and 66% for grasses, and 63 to 67% for legumes.

Key words : natural pastures, Algeria, floristic composition, chemical composition, *in vitro* digestibility.

INTRODUCTION

Livestock feed is considered, after genetics, to be the most effective means of increasing animal production, but in Algeria it is undeniably one of the major constraints on the various livestock production systems. However, modern livestock farming needs to be well thought-out in order to adapt forage availability to animal requirements.

In Algeria, the availability of forage units and nitrogenous matter is insufficient, or a chronic deficit is recorded every year (3 to 4 billion FU depending on the year). Forage areas are estimated at 1,988,519 ha but represent only 2.6% of the area used for agriculture (MADR, 2014). Faced with this situation, rapid and sustainable solutions are needed.

Natural fodder such as natural grasslands are a significant source of energy and nitrogen for ruminants, despite the relentless reduction in their surface area, and the low level of human intervention to maintain and improve them.

Grasslands provide a number of important ecosystem goods and services. First and foremost, they provide fodder for livestock (Bailey et al., 1996; Schauer et al., 2005). They are also important food sources for insects. They help maintain soil moisture, purify water and prevent flooding, and their roots hold the soil in place.

Restoring the importance of grasslands could help reduce the chronic forage deficit that Algeria has been experiencing for decades. One solution would be to study these grasslands and their forage potential, so as to be able to improve them and integrate them into livestock feed.

This study is part of this approach. The aim is to study the forage potential (botanical composition and yields) and nutritional potential (chemical composition and *in vitro* digestibility) of the dominant species of a natural grassland in the central region of Algeria.

MATERIAL AND METHOD

1. Study area and climatic characteristics

The study was carried out in Dellys (Daira headquarters) in the wilaya of Boumerdes. Dellys is a coastal town 105 km east of the capital Algiers.

Its climate is Mediterranean, with cold, wet winters and hot, dry summers. The wet season lasts 08 months, from October to May, and the dry season lasts 04 months, from June to September.

During the study year, cumulative rainfall was 595 mm, with significant seasonal variation, and temperatures also varied between seasons, with a minimum of 8°C in January and a maximum of 37°C between July and August.

2. Study plot and sample collection

The study plot is located in the Thouabet locality (Dellys commune). It covers an area of 0.65 ha. Samples were taken in spring (early April, 2021). Each sample consists of a mixture of legumes, grasses and other plants. The development stage of the various species sampled is shown in Table 1.

Our samples are taken using the simplified method of Theau et al, (2010). This technique limits the time required to establish a vegetation survey on a given plot. The sampling principle is in the form of frames, which limits the number of observations while increasing the surface area studied. We decided to take 7 samples, using a 50 cm square frame (0.25m^2), along a transect zigzagging across the entire plot, as shown in Figure 1.

In each frame, the frequencies and abundances of the dominant species are determined using the scoring system suggested by De Vries (1959). The contents of each frame were then weighed to obtain the fresh yield, then dried for 48h at 60°C to obtain the dry matter (MS%) and dry yield (Qx/ha). Finally, we proceeded to separate the dominant species in each frame. After identification, we mixed the contents of the 7 frames. In total, we were able to count 7 samples for the most abundant species (Vulpia ciliata, Alopercurus pratensis, Bromus hordaceus, Avena sterilis, Hedysarum, Melilotus indica). All species were ground with a 1 mm grid hammer mill, preserved and labelled for later analysis 3.

Table 2.Development stage of species sampled.

Sample type	Dominant species	Spring sampling
Grasses	*Vulpia ciliata*	Emergence
	Alopecurus pratensis	
	Bromus hordaceus	
	Avena sterilis	
Legumes	*Hedysarum flexuosum*	Flowering
	Melilotus indica	
Others	*Chrysantemum myconis*	Flowering
	Galactites tomentosa	
	Stachys ocymastrum	
	Centaurea pullata	
	Convolvulus tricolor	
	Daucus carota	
	Malva parviflora	

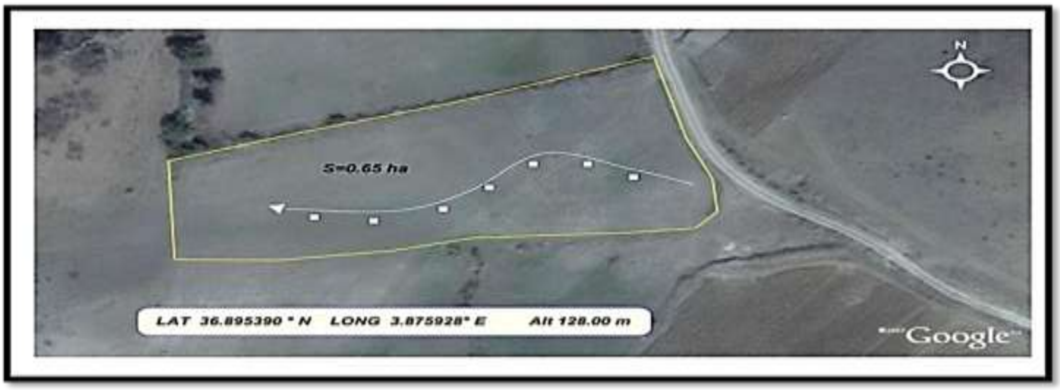


Figure 11.Location and method of sampling in the plot (Google earth, 2018).

3. Analysis and calculations

- For the agronomic study, frequency and abundance are calculated using the following formulas:

Abundance rate (B%) = (sum of scores for all frames/ n x 6).

Frequency (F) = number of times/ number of frames.

Frequency rate (F%) = (F / number of frames) x 100.

n : the number of frames used = 7

- Analysis of the chemical components: dry matter (DM); organic matter (OM); mineral matter (MM) and total nitrogenous matter (TNM) are carried out to AOAC (1990) standards, and total wall (NDF), lignocellulose (ADF) and lignin (ADL) by the sequential method of Van Soest and Wine (1963).

- Dry matter digestibility (DMD) and organic matter digestibility (OMD) are measured *in vitro* using the method of Tilley and Terry (1963). *In Vitro* digestibility of OM (per 100g DM) is calculated according to the following equation:

OMD (%) = (OM of sample - ROM) / OM of sample

With : ROM = Residual Organic Matter

The results are then corrected against a control (alfalfa hay of known digestibility).

All results were statistically processed by STATISTICA (version 6, 2003).

RESULTS AND DISCUSSION

1. **Floristic composition and biomass production**

2. According to the results obtained (Table 2), legumes were present at a rate of 50%, represented mainly by two species, *Hedysarum flexuosum* with an abundance rate of 28.6%, and Melilotus indica with 21.4%.

For grasses, the abundance rate is 35.71%, distributed between Avena sterilis (21.43%), Alopecurus pratensis (7.14%), Vulpia ciliata (4.76%) and Bromus hordaceus (2.38%). Avena sterilis is the representative grass species, with a frequency rate of 100%. On remarque également la présence d'autres espèces appartenant à différentes familles botaniques nommées dans la présente étude « autres » avec 14%, en grande partie occupée par l'espèce Galactites tomentosa avec une abondance de 4,76%. Table 2 shows the results of the frequency and abundance rates of the various dominant species on the plot during spring for the year under study.

Table 3.Frequency and abundance of dominant species in the Thouabet plot.

Genus	Dominant species	Frequency	Frequency rate	Total abundance	Abundance of each genus
Grass	***Vulpia ciliata***	**2**	**28,57%**	**4,76%**	**35,71%**
	Alopecurus pratensis	**3**	**42,85%**	**7,14%**	
	Bromus hordaceus	**1**	**14,29%**	**2,38%**	
	Avena sterilis	**7**	**100%**	**21,43%**	
Legume	***Hedysarum flexuosum***	**6**	**85,71%**	**28,57%**	**50%**
	Melilotus indica	**7**	**100%**	**21,43%**	
Others	***Chrysantemum myconis***	**2**	**28,57%**	**7,14%**	**14,28%**
	Galactites tomentosa	**5**	**71,43%**	**4,76%**	
	Stachys ocymastrum	**1**	**14,29%**	**2,38%**	
	Centaurea pullata	-	-	-	
	Convolvulus tricolor	-	-	-	
	Daucus carota	-	-	-	
	Malva parviflora	-	-	-	

According to Hubert and Pierre (2009), the dominant species (legumes or grasses) form what is frequently referred to as the "meadow base". These species ensure the biomass production of the plot. Ecological factors such as soil composition and climate have a major influence on the distribution of prairie species.

Among the most frequent species in Mediterranean regions, Avena sterilis provides the most ecological information. It is therefore an indicator species with a very wide ecological range (Kheddam and Adane, 1996).

Hedysarum flexuosum is a legume with an Ibero-North African distribution, endemic to north-central Algeria (Abdelguerfi et al, 1991), and thrives in regions with average rainfall of over 550 mm, in the humid and sub-humid bioclimatic zone (Abdelguerfi and Laouar, 1999).

According to some work carried out in Algeria, this legume is considered an excellent forage resource (Kadi et al, 2015).

According to Benjamin (2014), Melilotus indica is a forage plant of the legume family that has a wide natural range and grows in moderately saline areas.

Table 3 reports the fresh matter yield (RMF) and dry matter yield (RMS) of the various samples taken from the study plot.

Table 4.Yield (Qx/ha) of the Thouabet plot during spring.

Season	Frames	% DM at sampling	Fresh yield (Qx/ha)	Dry yield (Qx/ha)
Spring	1	14,63	328	48,00
	2	17,34	386	68,32
	3	14,21	366	52,00
	4	16,27	432	70,28
	5	19,17	240	46,00
	6	14,41	342	49,28
	7	10,32	370	38,20
Mean±SD		**15,19±2.80**	**353,14±60.41**	**53,15±11.84**

There was considerable variation between sampling points, with an average of 353.14 Qx/ha and 53.15 Qx/ha for fresh and dry yields respectively. DM content averaged 15.19%, with considerable inter-frame variability. DM yields depend on the floristic component of the meadow. They increase with wall content and plant height. Indeed, the results obtained by Lalmas and Bouchareb (2021) on the same plot show that *Hedysarum flexuosum* and Melilotus indica (legumes) represent the highest dry yields, thanks to the high weight and height of these species (103cm and 123cm respectively).

Huyghe et al (2015) report a ratio of 1 to 10 in the productivity of permanent grasslands between Portugal and Germany, mainly explained by soil and climate conditions, particularly the rainfall regime.

In Algeria, Salhi et al (2019) report that the fodder potential of the wasteland studied in the Chlef region stands at 38 Qx/ha, while DM content is 16.7 and 38.8%, which remain high compared with our results, due to the quality of the fodder produced. Adane and Kheddam (1996) found that grasslands on the Mitidja plain produced an average of 41 Qx DM/ha/year. The productivity of these grasslands is highly variable, ranging from 9 to 83 Qx DM/ha/year. According to these authors, these figures correspond to the quantity of forage harvested, and may be lower than the actual production of the grassland if it is under-exploited.

2. *In vitro* chemical composition and digestibility of the various species sampled

As mentioned above, the dominant and most representative species of the grassland were separated in order to determine their contribution to the forage value of the grassland.

Table 4 shows the results of the forage analysis and *in vitro* digestibility of the dominant species in the study meadow.

The results of the chemical composition show that there is a huge difference between the components, particularly for TN. These vary between 8% and 20% between the different species. For OM, they vary between 85 and 88%.

Table 5.Composition chimique et digestibilité In vitro des espèces dominantes de la prairie.

		% MS						%	
Species	**% DM**	**MM**	**OM**	**TN**	**NDF**	**ADF**	**ADL**	**DMD**	**OMD**
Melilotus indica	14,63	13,86	86,14	18,54	51,83	38,62	6,74	61,07	67,45
Hedysarum flexuosum	17,34	14,49	85,51	20,55	51,64	39,54	9,81	56,17	63,30
Vulpia ciliata	10,32	11,68	88,32	9,88	76,68	44,12	4,28	56,83	64,09
Avena sterilis	16,27	14,37	85,63	8,86	68,01	37,29	4,06	54,08	60,17
Alopecurus pratensis	19,17	14,42	85,58	11,89	51,35	39,34	3,99	56,18	66,38
Bromus hordaceus	14,41	12,6	87,4	11,77	73,89	43,94	4,61	56,49	62,44
Autres espèces	14,21	13,41	86,59	8,25	77,6	47,6	4,37	55,40	63,68
P value		**0,001**	**0,001**	**<0,0001**	**<0,0001**	**0,003**	**0,001**	**>0,05**	**>0,05**

Hedysarum flexuosum and Melilotus indica are richer in TN than the other species. These different results for DM and TN are explained by the species' botanical family.

Analysis of variance revealed a highly significant difference for TN ($p<0.001$), indicating a large difference in this component between species. This difference can be explained by the influence of the species' botanical family, which explains why *Hedysarum flexuosum* and Melilotus indica species have a high nitrogen content, unlike grasses such as Avena sterilis and Vulpia ciliata, which have a low TN content.

According to Hoden (1973), nitrogen in the ration stimulates the activity of the microbial population, in particular its cellulotic activity, and also increases the rate of degradation of the forage's parietal constituents. It thus helps improve forage digestibility.

On the other hand, OM content varies from one species to another. According to Coppen et al, (1974) and Duru (1992), this difference can be explained by the fact that the absorption of mineral elements by the plant ceases as from heading.

Total wall density (NDF) varies between 54 and 77% for Alopecurus pratenis and Vulpia ciliata species respectively.

Lignin composition is the parameter that differentiates species the most, with 4% for Alopecurus pratenis and 9.8% for *Hedysarum flexuosum*, i.e. more than double for the latter species.

This difference observed for NDF and ADL is confirmed by the analysis of variance, which shows a highly significant difference (P< 0.0001) between species of the same season.

According to Jarrige (1988), variable NDF contents can be explained by a different evolution of the leaf/stem ratio, given that stems contain more walls. Arab et al (2009) report that ADF fibers are generally linked to the digestibility and energy value of forage. Indeed, the higher the ADF content of the forage, the lower the digestibility and energy content.

The average DM digestibility of legumes is 58.62%, varying between 56.17 and 61.07% between the two species. For grasses, DIV averaged 56.07%, with values varying between 54.80 and 56.83%. For other species, digestibility averaged 55.40%. Legumes are logically more digestible than grasses and “other” species.

Grass digestibility ranges from 54.80% to 56.83% for Avena sterilis and Vulpia ciliata respectively, making the latter the most digestible species.

Demarquilly (1989) indicates that the large differences in digestibility between selected and native grass species (Bruinenberg et al, 2002) are due to two processes. The first is when biomass is the same, the share of the structural compartment is higher for species with a resource conservation strategy.

The other is when the structural compartment digestibility of these same species is lower than that of resource-capture species. Thus, the average values for grasses with capture and conservation strategies are 58 and 49% respectively (Al Haj Khaled et al, 2006).

Among legumes, Melilotus indica is more digestible than *Hedysarum flexuosum*. According to Arab et al (2009), high lignin content tends to reduce forage digestibility. The lower the lignin content, the higher the digestibility of the plant, i.e. the animal will consume more easily if the feed is rich in nitrogen and low in lignin. Lignin has an effect on plant utilization, binding with nitrogen and preventing nitrogen from being used by the animal (Chabaca, 2004).

Rodrigues et al (2007), indicate that differences in digestibility between species depend on the time at which they are exploited. This is because plant phenology differs from one species to another.

Overall, the forage on this plot is considered average to good. It is the equivalent of Algerian alfalfa with an average digestibility of around 59% (Alane, 2017).

For OM digestibility, it was calculated from residues after passage through a muffle furnace according to the equation of Minson, Raymond and Harris (1960). Grass OMD varies between 58 and 66% for Bromus bordaceus and Alopecurus pratensis respectively. On average, the OMD of these grass species is 62%. Demarquilly (1989); Facker and Minson (1981), agree that in spring, digestibility decreases essentially in relation to the proportion of stems in the harvestable biomass, the latter being less digestible. For grasses, such a reduction is also observed at the leafy stage (Ducrocq and Duru, 1994).

Among legumes, Melilotus indica has the highest OMD at 67%, while *Hedysarum flexuosum* is less digestible at 60%. The average OMD is 64%. For the “others”, comparable BMDs were obtained, ranging from 53 to 78%, with an average of 63.68%.

According to BAUMONT et al (2007), the digestibility of organic matter is closely linked to the indigestible wall content of the forage, with grasses and legumes forming part of the same relationship. Wall content and digestibility evolve in the plant according to time-dependent processes. Over the course of a growth cycle, plant wall content increases with plant growth, and wall digestibility decreases with tissue ageing (review by DURU et al, 2008).

Ballard (2009b), notes that an increase in parietal constituents would imply a loss of 2 to 3 points in OMD. Similarly, Van Soest (1982) shows that cell wall richness has a negative influence on digestibility.

CONCLUSIONS AND RECOMMENDATIONS

At the end of this study, the following findings emerged:

The floristic composition of the Thouabet meadow is based on legumes, with a rate of 50% for spring (2021). The legume family is mainly represented by *Hedysarum flexuosum* and Melilotus indica for this plot. As for grasses, Avena sterilis remains the most abundant on the plot.

In terms of yield, the dry biomass produced by the same plot during the spring is 53 Qx/ha, which is lower than in previous years. With a dry matter content at harvest of 15%.

The species with the highest TN content was Hydesarum flexuosum, with 20.55% DM. In terms of chemical components, NDF, ADL and TN content show a difference between species.

However, Hydesarum flexuosum is also the richest in lignin, with a content of 9.81% DM.

The *in vitro* digestibility of the different species in our plot is higher for legumes (58.62%) than for grasses (56.07%), but overall the grassland is almost similar and shows no significant difference between species, as shown by the analysis of variance of the parameters studied ($p > 0.05$).

On the other hand, OMD also represents significant levels for practically all 6 species and “others”, with a maximum level for Melilotus indica (67.45%).

These results show that, despite their low maintenance (neither worked nor fertilized), natural grasslands have considerable fodder potential and can play a key role in diversifying and improving fodder supplies for ruminants.

Further studies are needed to better characterize this dormant resource, and improvement actions (introduction of species, fertilization, etc.) remain essential to enhance the nutritional value of these grasslands.

REFERENCES

Abdelguerfi A. et Laouar M., 1999. Autoécologie et variabilité de quelques légumineuses d‟intérêt fourrager et /ou pastoral. Possibilité de valorisation en région méditerranéenne. Pastagense Forragens, 20, 81-112.

Abdelguerfi-Berrekia R., Abdelguerfi A., Bounaga N. et Guittonneau G G., 1991. Répartition des éspèces spontanées du genre Hedysarum selon certains facteurs du milieu en Algérie.Fourrages, 1991, 126, 187-207.

Adane N., Kheddam M., 1996. Contribution à l'étude phytoécologique des mauvaises herbes des cultures pérennes dans la plaine de la Mitidja. 1. Aspect floristique.

Alane F., 2019. Recherche sur des espèces spontanées du genre Medicago d‟Algérie : Caractéristiques phénologiques,biométriques et nutritionnelles. Thèse doctorat, Ecole Nationale Supérieure Agronomique El-Harrach, Alger.136p.

Arab H., Haddi M.L., Mehennaoui S., 2009. Evaluation de la valeur nutritive par la composition chimique des principaux fourrages des zones aride et semi-aride en Algérie.Sciences et Technologie C- N 30, pp.50-58.

Bailey, D. W., Gross, J. E., Laca, E. A., Rittenhouse, L. R., Coughenour, M. B., Swift, D. M., et Sims, P. L. (1996). Mechanisms that result in large herbivore grazing distribution patterns. Rangeland Ecology et Management/Journal of Range Management Archives, 49(5), 386–400.

Ballard V., 2009b. Analyse des valeurs alimentaires de la luzerne déshdratée en fonction du temps de pré fanage. Coop de France Déshydratation, 1-34.

Baumont R., Aufrère J et Meschy F. (2009). La valeur alimentaire des fourrages : rôle des pratiques de culture, de récolte et de conservation. Fourrage, 198, 153-173.

Baumont R., Dulphy J P., Sauvant D., Meschy F., Aufrère J., Peyraud J L., 2007. Chapitre 8. Valeur alimentaire des fourrages et des matières premières : tables et prévision", Alimentation des bovins, ovins et caprins, Tables INRA 2007, Editions Quae, 149-179.

Benjamin L., 2014. Légumineuses et graminées couvre- sol et fourragères tropicales.

Bruinenberg M.H., Valk H., Korevaar H,. Struik PC., 2002. Facteurs influant sur la digestibilité des fourrages tempérés des prairies semi-naturelles : un bilan .Première publication:11 décembre 2002.https://doi.org/10.1046/j.1365-2494.2002.00327.x

Chabaca R., 2004. Valeur azotée chez Ovis ariés des pailles de Triticum durum traitées ou non à l‟ammoniac : facteurs déterminants. Thèse de doctorat d‟état en sciences agronomiques. Institut national agronomique, El –Harrach (Alger). 130p.

Coppen, A., Brooksbank, B. W., Eccleston, E., Peet, M., & White, S. G. (1974). Tryptophan metabolism in depressive illness. *Psychological Medicine*, *4*(2), 164-173.

De Vries D.M., Boer T.A., 1959. Methods used in botanical grassland research in the Netherlands and their application, Herbage Abstracts, 29, 1.

Demarquilly C., 1989.The feeding value of forages. XVI International Grasland Congress. Nice,France,pp 1817-1823.

Ducrocq H., DURU M., 1994. In vitro digestibility of green leaves of Dactylis glomerata in relation to leaf demography, Proc. 15'" Gen. Meet. Europ. Grass/. Soc., 6-9 Juin,157-161.

Duru M., 1992. Diagnostic de la nutrition minérale de prairies permanentes au printemps. I. Établissement de références. INRA, centre de recherches de Toulouse, station d‟agronomie, BP27 - 31326 Castanet-Tolosan, France (Reçu le 10 octobre 1990; accepté le 16 janvier 1992).

Duru, M., Cruz, P., et Theau., J.P. (2008). Un modèle générique de digestibilité des prairies semées et permanentes pour raisonner les pratiques agricoles. Revue trimestrielle Fourrages, 193, 79-102.

Hoden A., 1973, Valeur alimentaire des fourrages pauvres pour les ruminants. Effet de la complémentation azotée, Bull. Techn . CRZV INRA THEIX ; pp127.

Hubert F. et Pierre P., 2009. Guide pour un diagnostic prairial. 241p.

Huyghe C., Peeters A., De Vliegher A., 2015. La prairie en France et en Europe. Paper presented at the Colloque présentant les méthodes et résultats du projet Climagie (métaprogramme ACCAF), Poitiers, France.

Jarrige R., 1988. Alimentation des bovins, ovins et caprins. INRA Editions, 471p. 2-7380- 0021-5. ⟨hal-02854912⟩.

Kadi S.A., Guermah H., Mouhous A., Djellal F. and Berchiche M., 2015. Sulla flexuosa (Hedysarum flexuosum) : an not well-known forage legume of the Mediterranean coast. Actas AEL nr. 6. EUCARPIA international Symposium on Protein Crops, V Meeting AEL,Pontevedra,Spain, May 4-7 2015,127-128.

Lalmas H., Bouchareb Y., 2021. Caractérisation floristique et production de biomasse de prairie permanente de la région centre. Mémoire de Master ; Ecole Nationale Supérieure Agronomique El-Harrach Alger, 87p.

MADR., 2014. Ministère de l‟Agriculture et de Développement Rural, Statistiques agricoles.

Minson D. J., Raymond W.F., Harris C.E., 1960. Studies in the digestibility of herbage. VII. The digestibility of S37 cocksfoot, S23 ryegrass and S24 ryegrass. J. Brit. Grassl. Soc. , 15, 174-180.

Rodrigues A., Andueza D., Picard F., Cecato U., Farruggia A., Baumont R., 2007. Valeur alimentaire et composition floristique des prairies permanentes : premiers résultats d‟une étude conduite dans le Massif Central. Renc. Rech. Ruminants, 14, 241244.

Salhi H., Meziane M., Ali-Benamara B., Bensaiid A., Noura A., 2019. La composition floristique et la productivité de l'herbe dans la plaine du moyen cheliff. 3éme séminaire international sur les pâturages, les parcours et l'alimentation des animaux dans le bassin méditerranéen. Recueil des résumés du SIPPABM 2019, 29p.http://sipabm2019.sciencesconf.org.

Schauer, C. S., Bohnert, D. W., Ganskopp, D. C., Richards, C. J., et Falck, S. J. (2005). Influence of protein supplementation frequency on cows consuming low-quality forage: Performance, grazing behavior, and variation in supplement intake. Journal of Animal Science, 83(7), 1715–1725.

Soest, P. V., et Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the Association of Official Analytical Chemists, 50(1), 50-55.

Soest, P. V., et Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. Journal of the Association of Official Analytical Chemists, 50(1), 50-55.

Theau J.P., Cruz P., Fallour D., Jouany C., Lecloux E., Duru M., 2010. Une méthode simplifiée de relevé botanique pour une caractérisation agronomique des prairies permanentes. Fourrages, 201, 19-25.

Tilley J.M.A., Terry R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. Grass and forage science, 18 (2), 104-111.

Van Soest P.J., 1982. Nutritional Ecology of the Ruminant. Cornell University Press.

NUTRITIVE VALUE OF SOME SPECIES OF THE ALGERIAN STEPPE (TIARET) PROTECTED BY EXCLUSION

Chafia CHIBANI[1] , Hanane RAMI[2], Brahim HADJSMAIL[3] , Douaa TOUMI[2], Issam BELAID[3] and Rabeha CHABACA[2]

[1]ENS, Kouba. Algeria
[2] ENSA, Alger. Algeria
[3]CRSTRA, Biskra. Algeria
Corresponding author e-mail : chafia.chibani@g.ens-kouba.dz

ABSTRACT

Our work is to study the quality of fodder resources of nutritional supports traditionally used by sheep. The study site is located in Tiaret, which is considered as a reception area for flocks and more precisely the M'kimen range. It is part of the High Plains steppes southeast of the city of Tiaret at an average altitude of 1,000 m with an area of 35,000 ha. The studied area offers more or less favourable conditions for the existence of a characteristic spontaneous flora which represents the available food source for the animals. The floristic study allowed us to identify 32 species belonging to different families represented by herbaceous and especially perennial plants. The analysis of the forage, of the total wall (AFNOR, 1985; Van Soest and Wine, 1967) and of the composition in phenolic substances by SPIR (Bertrand, 2002) of 7 samples among the 32 highlighted the great variability in chemical components between these different species. The DMD and OMD measured *in vitro* (Tilley and Terry, 1963) vary, for the extremes, respectively from 31 and 36% (*Artemesia Herba alba*) to 83 and 86% (*Peganum harmala*). These extreme values are explained by the extreme contents of total phenols of 9.3 and 5.0% as well as by the respective contents of TN (10 and 23%) and ADL (20.7 and 5.2%) for these same species. These results need to be reinforced by the study of other species collected and to know their level of palatability.

Keywords: livestock system, steppe, forage analysis; phenolic compounds; digestibility.

INTRODUCTION

Grazing land is the world's most important agricultural activity. Algeria is no exception to this land distribution statistic. With a total surface area of 238 million hectares, 41 million hectares (18%) are devoted to agriculture, including 33 million hectares to pasture. The latter is distributed as follows: 87.7% is occupied by steppe rangelands and grazing land; 10.6% by fallow land; 1.6% by forage crops and 0.17% by permanent grassland (Nedjraoui, 2002; Rami et al, 2021). Man has taken advantage of these vast areas of terrestrial grazing land, depending on the ecological and pedoclimatic characteristics of the ecozones and the endemic or imported herbivore species, by devising adapted production systems (FAO and L'ILRI ,2011).

Thanks to their many benefits, grazing areas can account for between 10% and 44% of gross domestic product in some poor countries (AU, 2010). They provide a livelihood for nearly 3 billion people. Numerous subsequent studies show that grazing lands are also effective carbon sinks, largely offsetting the 18% of GHG emissions from livestock production (FAO, 2009; Gac et al, 2010; Dollé et al, 2013). Their contribution has reduced this percentage to 14.5% (FAO, 2013).

To respond to this concern, we need to deepen our knowledge of the country's pastures in their entirety, in particular their productivity and the nutritional value of plant species in our grazing areas, in anticipation of their improvement, while being vigilant about the consequences of our actions on global warming (GW).

The present work deals with the nutritional value of some species found after the fencing of a parcel of land located in the Tiaret region, considered to be a herding area, and more specifically the M'kimen grazing area. The region is renowned for its ancestral pastoral vocation, with livestock farming providing the bulk of the local population's income.

MATERIAL AND METHOD

The study site is considered to be a herding area, more specifically the M'kimen grazing area. It is part of the Hautes-Plaines steppes to the south-east of the city of Tiaret, at an average altitude of 1,000 m and with a surface area of 35,000 ha.

The area studied offers more or less favorable conditions for the existence of a characteristic spontaneous flora, which represents the available food source for animals.

The species were harvested in late spring (2019), corresponding to the vegetative period for annual plants, after a period of defensing.

We analyzed 7 dominant perennial species in this steppe rangeland. These species (Table 1) dominate alone or in combination, forming several vegetation units.

After harvesting, the samples were air-dried, then ground in a hammer mill to a 1mm grid and stored for analysis.

Forage analysis: organic matter (OM), mineral matter (MM) and total nitrogen matter (TN) content was carried out in accordance with AOAC (1990) recommendations. Total wall (NDF), lignocellulose (ADF) and crude lignin (ADL) were determined according to Van Soest (1963). Phenolic composition is determined by SPIR (Bertrand, 2002). DMD and OMD are measured *in vitro* (Tilley and Terry, 1963). *In Vitro* digestibility of OM (per 100g DM) is calculated according to the following equation:

Div OM (%) = (OM of sample - ROM) / OM of sample

With : ROM = Residual Organic Matter

The results are then corrected against a control (alfalfa hay of known digestibility).

Calculation and statistical analysis of results

The results were statistically analyzed by means of regression calculations between variables. Calculations were performed using S-PLUS software version 4-5e (1998).

Table 6. Systematics of the 7 species collected on the M'kimen trail.

Samples	***Common name***		***Vernacular name(*)***	***Order***	***Family***	***Genus***
	French	**English**				
Artemisia Herba alba	Armoise blanche, Herbe blanche, Absinthe du desert	Wormwood	Chih (LT) ; Zazaré, Horasani (M. Orient) ; Kaysoum (Maroc) ; Izreg (berbere); La'anah (hebreux)	Asterales	Asteraceae	Artemisia
Atriplex canescens	Arroche	Fourwing saltbush	Guetaf	Caryiphillales	Chenopodiaceae	Atriplex
Lygeum spartum	Sparte	Lygeum	Senagh (LT) ; Gdin (RT)	Cyperales	Graminée	Lygeum
Peganum harmala	Rue sauvage	Harmala peganum	Harmel (LT) ; Tifri (RT)	Zygophyllales	Zygophyllaceae	Peganum
Plantago albicans	Plantain blanchissant	Chilean plantin	-	Plantaginales	Plantaginaceae	Plantago
Reseda lutea	Reseda jaune	Yellou mignonette	Denb el kharouf	Pareatales	Resedaceae	Reseda
Stipa paviflora	Stipe à petites fleurs	Normon needlegrass	Halfa	Cyperales	Graminée	Stippa

(*) LT: local phonetic transcription; RT: regional phonetic transcription ;

In addition to their role as a food resource for animals, these species are also of great ecological and economic importance. In fact, they are used for honey production, medicinal purposes (sedative, narcotic, antiasthmatic, bactericidal, insecticide, cosmetic) or to make handicrafts (Table 2). Often, the same plant species is used twice or even three times (for condiments, aromatic and medicinal purposes).

Table 7.Special properties of harvested plants.

Species	**Known properties**
Artemisia herba alba	Medicinal; essential oils; flavoring for tea and other beverages; condiment; vermifuge
Atriplex canescens	Medicinal; condiment
Lygeum spartum	Handcrafted (Ropes, baskets, soles)
Peganum harmala	Hallucinogenic properties (witchcraft)
Plantago albicans	Medicinal against constipation and dysentery, melliferous
Reseda lutea	Melliferous
Stipa paviflora	Handcrafted, rope and basket making

RESULTS AND DISCUSSION

Chemical composition of samples taken

All the chemical composition results are shown in Table 3.

Table 8.Average chemical composition of the 7 species in the M'kimen range.

Species	DM %		In % DM				
		MM	OM	TN	NDF	ADF	ADL
Artemisia herba alba	93,60	12,82	87,18	10,30	68,80	49,80	**20,67**
Atriplex canescens	94,75	16,72	83,28	10,71	60,48	31,30	9,66
Lygeum Spartum	95,10	6,11	93,89	**8,61**	**82,28**	**52,78**	6,52
Peganum harmala	92,05	13,26	86,74	**23,46**	**30,70**	**19,83**	**5,17**
Plantago Albicans	93,55	20,74	79,26	13,10	54,73	38,59	9,73
Réseda lutea	93,05	22,06	77,94	14,20	40,79	29,02	7,15
Stipa parviflora	95,55	5,61	94,39	11,47	81,95	40,03	8,16

OM varied between 78 and 94% for *Reseda lutea* and *Stipa parviflora* respectively. This large difference between species can be explained by the large difference in MM content between certain species. Steppe plants are certainly soiled by small particles of soil, but more likely by sand stored in plant organs such as hairs, which offer an adequate holding structure.

The average TN content is 13%, with a very wide range between the 7 species, as shown by the minimum of 8.6% (*Lygeum Spartum*) and the maximum of 23.5% (*Peganum harmala*), i.e. almost 3 times higher. However, this parameter remains within the range of TN contents of hay in Algeria (Chibani, 2013).

Sequential wall analysis in Table 3 shows a low average NDF total wall content of 60%, but the differences between the minimum of 31% (*Peganum harmala*) and the maximum of 82% (*Lygeum Spartum*) remain significant, with almost 3 times as much as for TN.

Lignin content appears to be the parameter that most discriminates the wall content of the species harvested, with a maximum of 21% and a minimum of 5.2% of DM, giving an average of 9.6%. This is the case for *Peganum hermala*, with the lowest ADL content, and *Artemisia herba alba*, with the highest. Lignin is known to be the main compound influencing the depreciation of indigestibility and digestibility of forages.

It has been reported that increasing temperature (the case in our study region) favors lignification. Indeed, rising temperatures can induce phenological (advanced flowering) and physiological (thicker cell walls and increased lignin concentrations) changes in plants

(Kering et al, 2011), and also favors species turnover by favoring taller, slower-growing species that invest more energy in structural support and defense, and less energy in leaf growth (Waghorn and Clark, 2004).

In general, fiber content is due to variations between plant species, maturity and environmental conditions. Similar results were reported by Boufennara et al (2012), who found that the high fiber content (ADF and lignin) of some forage species could be partly explained by ecological conditions - high temperature and low precipitation. This tended to increase the cell wall fraction and decrease the soluble content of the plants. This observation applies to our case (arid bioclimatic stage) where rainfall is low (around 200mm on average) and temperature high from spring to autumn (over 36°C on average).

In vitro digestibility of harvested species

Digestibility is considered the second step in forage evaluation after forage analysis. Variations in OMD results can be attributed to several factors, but mainly to differences in the chemical composition of feeds, (Tufarelli et al, 2010). In addition, values for total nitrogen matter, fiber content and digestibility are indicators of the nutritional quality of feeds for ruminants (Andualem et al, 2016).

The *in vitro* digestibility of our 7 harvested samples is reported in Table 4. Our OMD measurement results show an average of 56.5%, but ranges from 33% for *Artemisia herba alba* to 83% for *Peganum hermala*.

The lower lignin content (5%) and higher TN content (23%) of *Peganum hermala* could partly explain this extremely high digestibility result.

It can be seen that the lower the fiber content (Table 3), the higher the digestibility (*Peganum harmala*). These results can be explained by the total tannin content in the walls of certain species (Table 4). In fact, these components, even in a plant rich in OM, have a negative effect on OM digestibility, as shown by the *Artemisia herba alba* species with an OMD of only 33.4%.

In order to better understand and validate our observations, we established regressions between dMO and the different chemical components measured. Table 5 shows the results obtained.

The TT component was found to have a negative effect on OMD, accounting for 75% of its variations. Combined with ADF, the two parameters explain 96% of OMD ($p \leq 0.001$).

Tannins are said to form stable complexes with product proteins, insoluble in the rumen medium, and a large proportion of TN is thus rendered unavailable to the action of digestive enzymes (Larwence, 1983; Chabaca, 2004). These species confirm our observation that less total tannin favours a higher OMD (*Peganum* with 86%) and vice versa for *Lygeum* and *Stipa parviflora* (around 36%).

Foguekem et al (2011) suggested that OMD is strongly influenced by the amount of fiber represented by the levels of cellulose, hemicellulose and lignin in plant tissues. The results of our study are also in agreement with those of Ghulam et al (2017) who observed a negative correlation of OMD with NDF, ADF and lignin. In our study, ADF explained the most negative relationship with OMD (R^2 =0.92).

Table 9. Phenolic compounds and average in vitro digestibility of the 7 species of the M'kimen range.

Support	**Total phenols (%DM)**	**Total tannins (%DM)**	**Substances Total phenolics (%DM)**	**DMD(in %)**	**OMD(in%)**
Artemisia herba alba	4,79	4,50	9,29	33,20	39,47
Atriplex canescens	4,16	1,83	5,99	57,20	75,74
Lygeum Spartum	0,21	5,43	5,64	33,00	36,55
Peganum harmala	3,02	2,04	5,06	75,30	83,00
Plantago Albicans	1,24	5,68	6,92	48,00	51,50
Réseda lutea	2,27	3,36	5,63	72,00	78,40
Stipa parviflora	2,31	5,20	7,51	39,50	44,70
TP : Total phenols, TT : Total tannins, STP: Substances Total phenolics					

Table 10.Regression models between dMO measured in vitro and the chemical components of the 7 species studied.

Explanatory variables	**Equations**	**R^2**	**P value**	**RSE**
TT	**OMD= -11,267TT+105,59**	**0,75**	**0,01**	**11,36**
ADL ; TT ; TN	**OMD = -1,443ADL10,37TT+0,0002MAT+111,79**	**0,88**	**0,01**	**9,00**
MM ; TT	**OMD = -10,11 TT+0.89 MM+84.44**	**0,823**	**0,03**	**10,76**
ADF ; TT	**OMD = -1.282 ADF-4.14 TT+120.86**	**0,96**	**0,001**	**4,91**
ADF	**OMD = -1,7208ADF+120,67**	**0,92**	**0,0006**	**6,41**
ADF ; TN ; MM	**OMD = -2,133ADF-1.23MAT+0.067MM**	**0,95**	**0,018**	**6,60**
TT ; ADF ;MM	**OMD = 0,33 MM-4,31 TT-1,173ADF +112,86**	**0,97**	**0,008**	**5,00**
R^2= coefficient of determination; P value= 95% probability; RSE= residual standard error of the model				

CONCLUSIONS

Our data set reveals how different forage plant species growing in the same steppe rangeland can vary in their nutritional value for sheep flocks in particular and ruminants in general. Some forage plant species were highly nutritious, containing high concentrations of protein and low concentrations of fiber and lignin, but rich in total tannins. The characterization of forage qualities through laboratory trials is an essential step in long-term programs aimed at improving forage availability in pastoral and agro-pastoral environments. Experimental studies of the chemical composition of forage species, especially their total tannin content, have shown that restoring steppe ecosystems through fencing is a highly effective strategy, as several species have recorded good forage values.

Further work is needed to elucidate the effects of global environmental change on plant nutrient values, and also, through observations via surveys of livestock farmers, to find out whether or not these species are palatable to grazing animals.

REFERENCES

AFNOR, 1985 . Association française de normalisation (Afnor). Recueil de normes françaises. Méthodes d'analyses françaises et communautaires. Aliments des animaux. 2e édition. La Plaine Saint-Denis (France) : Afnor, 1985.

Andualem D., Negesse T., & Tolera A. 2016. Chemical composition, in vitro organic

Bertrand D, 2002.La spéctroscopie proche infrarouge et ses applications dans les industries alimentaires animales .INRA, Prod.Anim, 15, 3, 209-219.

Boufennara, S., Lopez, S., Bousseboua, H., Bodas, R., & Bouazza, L,2012. Chemical composition and digestibility of some browse plant species collected from Algerian arid rangelands. *Spanish Journal of Agricultural Research*, *10*(1), 88-98. https://doi.org/10.5424/sjar/2012101-134-11

Cameroon. *Scientific Research and Essays*, *6*(17), 3577-3583.

Chabaca R, 2004. Valeur azotée chez *Ovis ariés* des pailles de *Triticum durum* traitées ou non à l'ammoniac : facteurs déterminants. Thèse de doctorat d'état en sciences agronomiques. Institut national agronomique, El –Harrach(Alger). 176p.

Chibani C., 2013. Eléments princeps de la valeur alimentaire des fourrages algériens pour le ruminant : Modèles de prédiction. Thèse de doctorat en sciences agronomiques. Options : Production Animales, INA, El –Harrach(Alger). 180p.

Clark DA et GC Waghorn , 2004. Feeding value of pastures for ruminants ; DOI: 10.1080/00480169.2004.36448

composition and in vitro digestibility of Appennine pasture plants using yak (Bos grunniens)

Dolle JB, Faverdin P, Agabriel J, Sauvant D, Klumpp K ,2013. Contribution de l'élevage bovin aux émissions de GES et au stockage de carbone selon les systèmes de production. Fourrages, (215), pp.181 - 191.

FAO ,2013. Tackling climate change through livestock : A global assessment of emissions and mitigation opportunities, Rome, 139 p.

FAO 2009 . Review of evidence on drylands pastoral systems and climate change. Implications and opportunities for mitigation and adaptation. Rome. 50 pages.

FAO and ILRI , 2011. Global livestock production systems. Rome,Food and Agriculture Organization of the United Nations (FAO) and International Livestock Research Institute(ILRI), 152 pp.

Foguekem, D., Tchamba, M. N., Gonwouo, L. N., Ngassam, P., & Loomis, M, 2011.

Gac A, Dollé JB, Le Gall A , Klumpp K , Tallec T, 2010. Le stockage de carbone par les prairies : Une voie d'atténuation de l'impact de l'élevage herbivore sur l'effet de serre. Institut de l'Elevage – INRAe. Collection l'Essentiel. hal-02824535 12p.

Ghulam A,Shakeel A,Ashfaq A,Wajid N,Zartash F,Sajjad H,Muhammad H,Muhammad A K,Mirza H,Shah F,Kenneth J.B,Gerrit H , 2017. Quantification the impacts of climate change and crop management on phenology of maize-based cropping system in Punjab, Pakistan ? Agricultural and Forest Meteorology , Elsevier. https://doi.org/10.1016/j.agrformet.2017.07.012

Kering, M., Butler, T., Biermacher, J., & Guretzky, J. A. 2012. Biomass yield and nutrient

Larwence A, 1983 . Recherches sur les facteurs limitants de la faible valeur nutritive des marcs de raisin pour le ruminant : procédés d'amélioration ; Thèses de doctorat d etat es sciences ; Universite de Dijon ; 248p.

matter digestibility and kinetics of rumen dry matter degradability of morphological fractions

Nedjraoui D 2002. Les ressourcespastoralesenAlgérie.DocumentFAOwww .FAO.org /ag / agp / agpc /doc / counprof / Algeria / Algerie .htm vertigo revue org/5375

Nutritional status of forage plants and their use by elephant in Waza National Park,

of stinging nettle (Urticasimensis). Adv. *Biol. Res*, *10*, 183-190.

Rami H, Chibani-Khetib C, Medjadbi M et Chabaca R 2021. Contribution à l'étude de la valeur fourragère des prairies permanentes algériennes. Caractérisation botanique et agronomique en région centre. *Livestock Research for Rural Development.* http://www.lrrd.org/publiclrrd/proofs/LRRD3307/cont3307.html

removal rates of perennial grasses under nitrogen fertilization. *BioEnergy Research*, *5*, 61-70.

rumen fluid or faecal extract as inoculum source. *Asian-Australasian Journal of Animal Sciences*, *23*(12), 1587-1593.

S-Plus Statistics 1998 4-5^{e} edition, Tokyo

Tilley JMA et Terry RA, 1963. A two stage technique for the in vitro digestion of forage crops. *Journal of the British Grassland Society* 8: 104-108.

Tufarelli, V., Cazzato, E., Ficco, A., & Laudadio, V, 2010. Evaluation of chemical

UA: Union Africanize 2010. Policy framework for pastoralism in Africa:Securing, Protecting and Improving the Lives, Livelihoods and Rights ofPastoralist Communities.Addis Ababa, Ethiopia 44p.

Van Soest P J., Wine R H., 1967. Use detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents. Journal of the Association of Official Analytical Chemists, 50, 50-55.

Waghorn GC, Shelton ID, McNabb WC and McCutcheon SN, 1994. Effects of condensed tannins in *Lotus pedunculatus* on its nutritive value for sheep. 2. Nitrogenous aspects. The Journal of Agricultural Science, Volume 123, Issue 1, pp. 109 – 119.

Yahiaoui A, 2011. Nutritionnel pour *Ovis aries* de différents supports alimentaires en zones agro-pastorales de l'ouest Algérien. Thèse de doctorat en Sciences Agronomiques, Option : science animales, ENSA EL Harrach (Alger), 129p.

IDENTIFICATION AND QUANTIFICATION OF NARINGENIN IN DIFFERENT TYPES OF HONEY FROM THE MOSTAR REGION USING HPLC

Amna BIJEDIC[1], Emil Dino FIDERSEK[2,3]

[1]Faculty of Agriculture and Food Science, University of Sarajevo, Bosnia and Herzegovina
[2]Faculty of Pharmacy, University of Sarajevo, Bosnia and Herzegovina
[3]*Hospital pharmacy - University Clinical Hospital Mostar*
Corresponding author: bijedic.amna@gmail.com

ABSTRACT

Honey has been used as a natural sweetener for thousands of years, produced by bees (*Apis mellifera*) from either nectar or honeydew. Its chemical composition is influenced by factors such as processing methods, botanical origin, environmental conditions, and seasonal changes. Among the many valuable components of honey are phenolic compounds, such as naringenin, a flavonoid with several health-promoting properties, including nephroprotective, anti-inflammatory, antioxidant, and anticancer effects. This study aimed to quantify the naringenin content in nine honey samples from three honey types—sage, heather, and meadow—collected from three distinct locations in the Mostar region: Rujiste, Bijelo Polje, and Podvelezje. High-performance liquid chromatography (HPLC) was employed for the quantification. The results revealed the presence of naringenin in most samples from Rujište and Podvelezje, with no naringenin detected in meadow honey from Bijelo Polje.

Keywords: honey, naringenin, HPLC, functional food, Mostar

INTRODUCTION

Polyphenols, which consist of more than one phenolic hydroxyl group attached to benzene rings, are widely distributed in plants and foods (Vermerris & Nicholson, 2006). These include various derivatives such as flavonoids, phenolic acids, and stilbenes (Shahidi & Naczk, 2004). In honey, polyphenols play a significant role as antioxidants, contributing to its therapeutic benefits, which include protection against oxidative stress and chronic diseases such as heart disease, cancer, and neurodegenerative disorders. Due to the therapeutic benefits of honey, it is used to treat several illnesses because of its various pharmacologically active components, especially flavonoids and phenolic compounds. Kaempferol, chrysin, quercetin, pinobanksin, pinocembrin, genistein, luteolin, apigenin, naringenin, hesperetin, gallic acid, p-coumaric acid, ellagic acid, ferulic acid, caffeic acid, syringic acid, and vanillic acid are a few of the flavonoids and phenolic chemicals (Zullkiflee et al., 2022). In food, polyphenols may contribute to bitterness, astringency, color, flavor, and odor (Pandey and Rizvi, 2009). Naringenin, a flavonoid in the flavanone subclass (Salehi et al., 2019), is known for its potent antioxidant and anti-inflammatory properties, along with its effects on cholesterol regulation, blood pressure, and cancer cell proliferation. Naringenin is primarily found in citrus fruits but has also been detected in honey. Evidence supports the analgesic activity of naringenin in a wide range of inflammatory conditions. Naringenin can control body lipids by possessing hypocholesterolemic and hypolipidemic effects and anti-estrogenic activities. Principally, flavonoids have been investigated and found to be a major regulator of tumor cell growth, EC migration, and angiogenesis. Besides, naringenin also regulates blood pressure and has antagonistic activities against inflammation (Manchope et al., 2022).

This study aimed to quantify the naringenin content in 9 honey samples: 3 different types of honey (sage, heather, and meadow honey) from 3 different locations in Mostar: Rujiste, Bijelo Polje, and Podvelezje. These locations are at varying altitudes, which may influence the phenolic content in honey.

The HPLC method was used to determine naringin for the sampled types of honey.

Figure 1. Map of localities from which samples were taken (Bijedic A.)

MATERIALS AND METHODS

Sample Preparation

Honey samples (5 g) were homogenized with 10 mL of deionized water. The pH of the solution was adjusted to 3 using HCl. Solid-phase extraction was performed using BOND Elut Plexa cartridges, which were conditioned with 5 mL of methanol followed by 5 mL of acidified deionized water (pH 3.5). Samples were passed through the sorbent at a flow rate of 2 mL/min. Analytes were eluted with 5 mL of methanol, evaporated to dryness, and redissolved in 1 mL of a mobile phase consisting of 0.05% TFA in water (90:10, v/v). The extracts were filtered through 0.45 μm filters before HPLC analysis.

Chromatographic Conditions

Chromatographic separation was conducted on a UHPLC system (Merck Hitachi, Germany) equipped with a pump (L-2160U), UV detector (L-2400U), autosampler (L-2200), and a temperature-controlled column compartment (L-2350U). Data was processed using EZ Chrom Elite software. The separation was performed on a Zorbax 300SB-C18 analytical column (150 mm x 4.6 mm; 3.5 μm, Agilent Technologies) at 25°C. A binary gradient system with 0.05% TFA in water (solvent A) and acetonitrile (solvent B) was applied (Table 1). Naringenin was detected at 285 nm with a total analysis time of 12.5 minutes.

Table 1. Gradient Program

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (mL/min)
0	90	10	0.8
10	30	70	0.8
10.1	90	10	0.8
12.5	90	10	0.8

Polyphenols were identified by comparing retention times with standards and confirmed through standard addition.

RESULTS AND DISCUSSION

Table 2. Naringenin Content in Meadow Honey (µg/g)

Location	Naringenin (µg/g)	SD
Podveležje	0.73	0.01
Rujište	9.80	0.01
Bijelo Polje	ND	ND
ND: Not detected		

Meadow honey from Podvelezje showed a significantly higher naringenin content than that from Rujiste ($p<0.05$), while no naringenin was detected in honey from Bijelo Polje.

Table 3. Naringenin Content in Sage Honey (µg/g)

Location	Naringenin (µg/g)	SD
Podvelezje	7.92	0.01
Rujiste	6.64	0.014
Bijelo Polje	5.34	0.01

In sage honey, Podvelezje showed the highest naringenin content, with significant differences observed between all localities ($p<0.05$).

Table 4. Naringenin Content in Heather Honey (µg/g)

Location	Naringenin (µg/g)	SD
Podvelezje	3.67	0.01
Rujiste	11.91	0.01
Bijelo Polje	3.84	0.01

Heather honey from Rujiste exhibited the highest naringenin levels, significantly higher than those from Podvelezje and Bijelo Polje ($p<0.05$).

CONCLUSION

The analysis demonstrated that heather honey from the Rujiste location contained the highest concentration of naringenin, whereas meadow honey from the Podvelezje location exhibited the lowest levels. Notably, naringenin was not detected in meadow honey from the Bijelo Polje location.

The findings indicate that honey from the Mostar region, particularly samples from Rujiste and Podveležje, represents a significant source of naringenin. This compound, known for its potent antioxidant and anti-inflammatory properties, underscores honey's potential as a functional food with considerable health benefits. The results support the role of honey in promoting health beyond its nutritional value as a natural sweetener. Future research should focus on elucidating the environmental and botanical factors that influence naringenin content in honey to further understand the variability in its functional properties.

REFERENCES

Manchope M.F., Ferraz C.R., Borghi S.M., Soares Rasquel-Oliveira F., Franciosi A., Bagatim-Souza J., Dionisio A.M., Casagrande R., Verri W.A. (2022): Therapeutic role of naringenin to alleviate inflammatory pain, Chapter 38 - Editor(s): Rajkumar Rajendram, Vinood B. Patel, Victor R. Preedy, Colin R. Martin, Treatments, Mechanisms, and Adverse Reactions of Anesthetics and Analgesics, Academic Press,P. 443-455,

Pandey KB, Rizvi SI. (2009): Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev, 270-8. doi: 10.4161/oxim.2.5.9498. PMID: 20716914; PMCID: PMC2835915.

Salehi, B.; Fokou, P.V.T.; Sharifi-Rad, M.; Zucca, P.; Pezzani, R.; Martins, N.; Sharifi-Rad, J.: (2019) The Therapeutic Potential of Naringenin: A Review of Clinical Trials. *Pharmaceuticals*, *12*, 11. https://doi.org/10.3390/ph12010011

Shahidi F., Naczk M. (2004): Phenolics in Food and Nutraceuticals, CRC PRESS, Boca Raton, London New York Washington, D.C, p.1-16.

Zullkiflee N., Taha H., Usman A. (2022): Propolis: Its role and efficacy in human health and diseases, Molecules, 27 (18), p. 6120, 10.3390/molecules27186120

Vermerris, W. and Nicholson, R. (2006): Phenolic Compounds and Their Effects on Human Health. In: Phenolic Compound Biochemistry, Springer, Dordrecht, p.2-2https://doi.org/10.1007/978-1-4020-5164-7_7

EFFECTS OF DIFFERENT NaCl DOSES on GERMINATION and EARLY SEEDLING STAGE of SOME RADISH (*Raphanus sativus* L.)

Tugce OZSAN KILIC[1*] *and Ahmet Naci ONUS*[1]

[1] *Akdeniz University, Faculty of Agriculture, Department of Horticulture, Turkey*
Corresponding author e-mail: tugceozsan@akdeniz.edu.tr

ABSTRACT

Seed germination and early seedling growth are the critical stage for species survival. Salinity affects the germination, seedling growth, and yield of several crop species, such as radish. The current study was carried out to reveal the effects of NaCl on seed germination and the early seedling stage of radish (*Raphanus sativus* L.). In order to study salinity stress on three radish cultivars, a laboratory experiment with a completely randomized design was conducted with three replications in the Department of Horticulture, Akdeniz University, Türkiye. To create salinity stress, sodium chloride (NaCl) at three levels of 0 (as control), 100, 200 and 300 mg L^{-1} were used. Results showed that there were statistical differences on final germination percentages (%), hypocotyl lengths, and root lengths in terms of cultivars. Accordingly, among the cultivars tested, the black radish variety showed better performance than the other cultivars regarding the parameters measured at different salinity levels.

Keywords: Radish, Salinity, Germination, Germination Percentage, Growth Parameters

INTRODUCTION

Throughout the world, radish (*Raphanus sativus* L.) is grown and consumed as a vegetable, making it one of the more significant species (Wang et al., 2013). It is well-known for its unique taste and fleshy root with a significant culinary component. It's rich in nutrients, including protein, carbs, crude fiber, and vitamin C (Lu et al., 2008). It also contains beneficial bioactive molecules such as isothiocyanate, polyphenols, and glucosinolates. Radish's characteristically strong taste comes from volatile isothiocyanates (Ghosh et al., 2014). Radish's ultimate yield and quality are determined by glucosesinolates, which also have a major effect on the vegetable's nutritional content and flavour (Kanjevac et al., 2021). As a species that is sensitive to salt, radishes are known to be inhibited in their growth by salinity. Stress due to salt has been shown to impact metabolic processes in seedlings and to produce chlorosis, morphological and anatomical abnormalities, and indications of nutritional inadequacy in matures in crops (Kanjevac et al., 2021). Numerous research findings demonstrated that salt stress has a detrimental impact on radish plant height, chlorophyll content, and fresh weight of the shoot and root.

The radish cultivars exhibited significant differences in germination and growth characteristics as a result of varying salinity treatments (Ghosh et al., 2014; Vishnu Priya et al., 2020; Kanjevac et al., 2021).

The most crucial stage of a plant's life cycle is the seed germination (Khan et al., 2000). The capacity of the seed to germinate in various environmental circumstances and to do so quickly and uniformly is important characteristics of radish, just like in the case of many other species. The germination and seedling growth phases of many plant species are very susceptible to salt stress (Khan et al., 2000). Normally, non-saline circumstances yield the maximum germination percentage, which then declines with increasing salt concentrations. The term "germination" refers to the rootlet's exit from the testa (Coopland and McDonald, 1995). Water intake initiates seed germination, whereas salt inhibits it (Othman, 2005). In salted circumstances, the seed is unable to germinate because of a reduction in water absorption, osmotically, and ion toxicity due to high concentration of Na and Cl ions around the seed (Murillo-Amador et al., 2002). Increasing salt concentrations delays the onset of germination, which not only prevents seeds germination but also extends the time needed for germination (Rahman et al., 2008).

Plant physiological and biochemical processes are impacted by salt stress, which lowers the biomass output (Ahmad and Sharma, 2012; Ahmad et al., 2012; Kanjevac et al., 2021). Plants are negatively impacted by salt stress throughout their whole life cycle. The ability of a species to withstand salt stress varies and is also dependent on the stage of plant growth (Ahmad and Sharma, 2012; Ahmad et al., 2012; Kanjevac et al., 2021). Compared to mature plants, seedlings, and immature plants are noticeably more vulnerable to salt stress. The demand to create crops that can withstand more salt has grown significantly in recent years as a result of global increases in salinity issues. In comparison to land without salt, radish yield is significantly reduced in saline soil. The technology involved in producing any crop, including radish, is complicated, and it gets much more under salinized conditions. Therefore, understanding how different salinities affect radish seed germination is crucial for achieving the ideal plant population and maximum output. Prolonging the germination period can be highly hazardous for a crop that is sown directly into the soil, as there is a greater chance of layer formation on the top layer of the soil, which could hinder or even completely prevent the emergence. Additionally, young seedlings and recently germinated seeds are particularly vulnerable to various pests and fungi (Cuartero and Fernandez-Munoz, 1999). For these reasons, it is necessary to improve the plants' resistance to salt to maximize the yield of cultivars grown in salted soils. Knowing the germination properties of the seeds at varying salt concentrations is crucial in this regard. The capacity of the seeds to germinate under salt-stress circumstances and their tolerance to salt during germination are investigated in present study. The seeds are from three different radish cultivars/types with varying salt concentrations. To serve the purpose, seeds of several radish cultivars are treated with salt concentrations of 0, (control), 100, 200, and 300 mg L^{-1}. After the experiment, germination percentage, final germination percentages (%), hypocotyl lengths (cm), and root lengths (cm) were measured and recorded.

MATERIAL AND METHOD

This study was designed to evaluate the germination characteristics of three different radish cultivars/types, namely black radish, radish Antep, and fındık radish, under laboratory conditions at the Department of Horticulture, Faculty of Agriculture, Akdeniz University in Turkey.

Preparation of seeds

The study was conducted in sterile cabins at every step. Following autoclave sterilization, glass petri dishes with a 90 mm diameter and filter papers within were employed. Seeds were surface sterilized for fifteen minutes in a 20% sodium hypochlorite solution. It was then ready for seeding in petri dishes after being washed four times with sterile distilled water. Each petri dish included ten seeds, and the study was conducted with three repetitions with three petri dishes included in each repetition.

NaCl Treatments

Three distinct concentrations of NaCl were employed for salinity applications (100, 200, and 300 mg L^{-1}). Pure water was utilized in the 0 (control) media, where NaCl was not added. Each petri dish received 10 ml of the liquids containing the control and all NaCl concentrations applied. Three different observations were obtained during the study's ten days. Among the characteristics recorded were the radicle length (mm), hypocotyl length (mm), germination percentage (%). Petri dishes were placed in completely dark conditions and allowed to germinate at 25±2ºC. After germination, the studies were carried out in the plant development chamber at the same temperature and with a light/dark photoperiod of 16/8 hours. The emergence of the radicle from the testa was considered as the basis for germination. Throughout the experiment, a small amount of liquid was supplied to moisten the seeds as needed, considering the filter papers' state of drying.

Germination percentage was calculated with down given formula:

Germination Percentage (%) = Germinated seeds / Total cultured seeds x 100 (Gosh et al., 2014)

Statistical Analyses

An entirely randomized factorial design with three replications was used to conduct the current experiment and the statistical software JMP version 5.0.1 (SAS Institute Inc., Cary, NC, USA) was used to analyse the data.

RESULTS AND DISCUSSION

The purpose of this study was to ascertain how salt stress affected the germination characteristics of radish cultivars/types. Positive (normal) germination was defined as the emergence of a 2 mm root segment from the seed coat. Figures 1 – 4 illustrate the variations' germination percentages, hypocotyl lengths, and root lengths in relation to the applied salt concentrations.

Germination percentage of Antep radish was adversely impacted by increasing the salt concentration. Black radish was the least impacted by the highest NaCl concentration regarding germination percentages. Fındık radish was heavily affected by the 200 mg L^{-1} NaCl concentration (Figure 1). Similar to Gosh et al. (2014), who examined the effects of salinity on the radish seed germination, development, and yield parameters, the current study also found that the effects of salt stress varied based on the radish cultivar/type. Additionally, the effects were found to depend on both the cultivar and the concentration of salt applied.

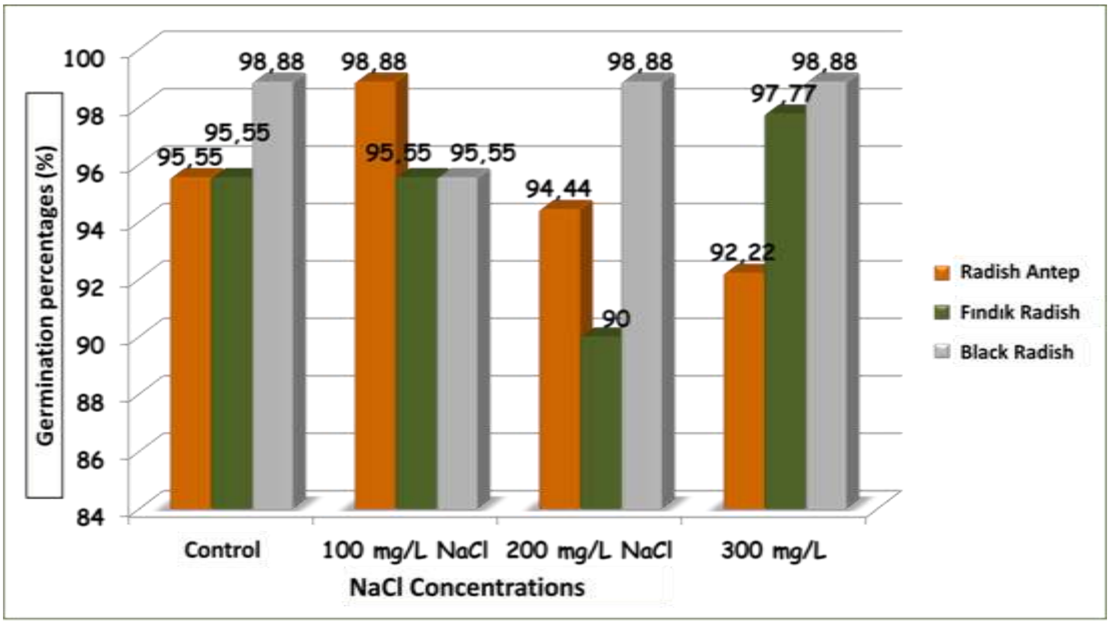


Figure 1. Germination percentages of three radish cultivars

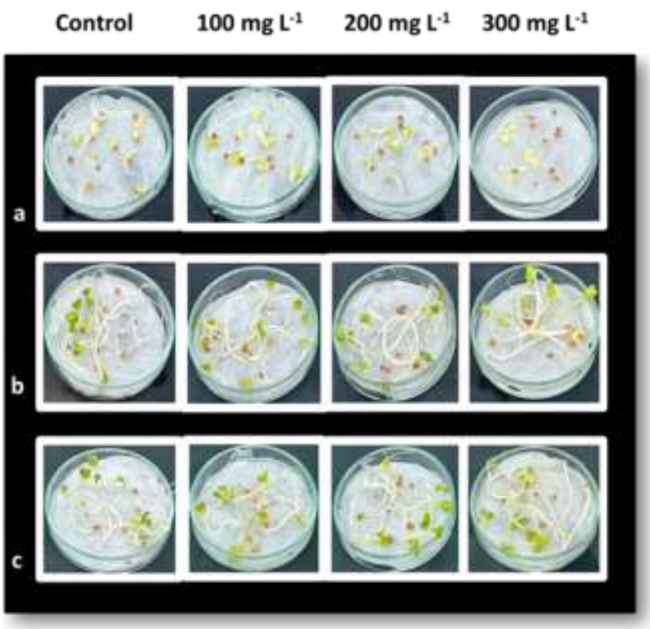


Figure 2. Germination characteristics of radish Antep under different NaCl concentrations; a. First measurement, b. Second measurement, c. Third measurement

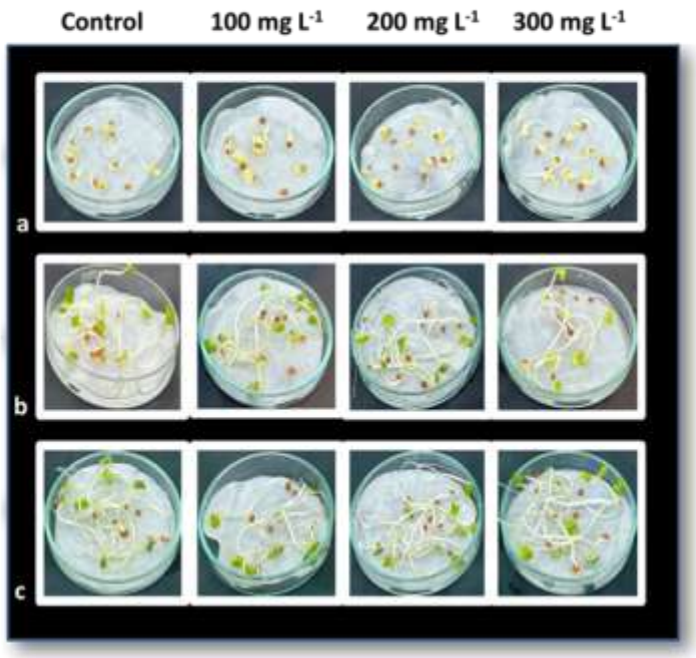


Figure 3. Germination characteristics of black radish under different NaCl concentrations; a. First measurement, b. Second measurement, c. Third measurement

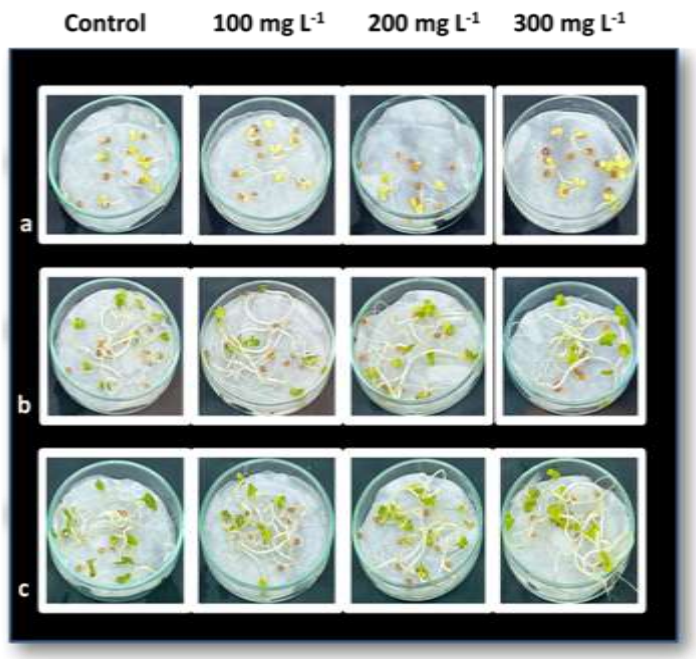


Figure 4. Germination characteristics of fındık radish under different NaCl concentrations; a. First measurement, b. Second measurement, c. Third measurement

Statistical differences in the number of seed germination within radish cultivars/types in terms of sodium chloride tolerance were observed upon evaluation of current findings as shown in Table 1. Therefore, it can be concluded that black radish has a better salt tolerance than fındık radish and Antep radish. Fındık and black radishes performed better than Antep radish when findings were evaluated in terms of root lengths, indicating statistically significant differences among the cultivars.

Table 1. Effect of NaCl concentrations on number of germinated seeds

Number of germinated seeds					
	NaCl Concentrations				Means of cultivars/types
Cultivar	Control	100 mg L^{-1} NaCl	200 mg L^{-1} NaCl	300 mg L^{-1} NaCl	
Antep Radish	9,66 ab	10,00 a	9,00 c	9,33 bc	9,50 *B*
Fındık Radish	9,33 bc	9,66 ab	9,00 c	10,00 a	9,50 *B*
Black radish	10,00 a	9,66 ab	10,00 a	10,00 a	9,91 *A*
Means of NaCl treatments	9,66 **AB**	9,77 **A**	9,33 **B**	9,77 **A**	
LSD Values	LSD cultivar= 0.313* LSD NaCl treat. = 0.361 LSD cult. x NaCl treat. = 0.626*				

*Different letters among cultivars and NaCl treatments denote significant differences (LSD test, $p<0.05$)

While the longest root length was recorded for black radish, the lowest root length was observed for radish Antep (Table 2). Cultivar/types differences in root length due to different NaCl concentrations were recorded in a number of previous researches. Similar results were also reported in some previous studies (Gupta et al., 1993; Zaman et al., 1995; Gosh et al., 2014).

Table 2. Effect of NaCl concentrations on root lengths

Root Length (mm)					
	NaCl Concentrations				Means of cultivars/types
Cultivar	Control	100 mg L^{-1} NaCl	200 mg L^{-1} NaCl	300 mg L^{-1} NaCl	
Radish Antep	63,72 c	78,27 abc	60,20 c	89,22 ab	72,85 *B*
Fındık Radish	88,38 ab	95,10 ab	79,94 abc	90,94 ab	88,59 *A*
Black Radish	93,88 ab	99,22 a	79,71 abc	73,44 bc	86,56 *A*
Means of NaCl treatments	82,00 **AB**	90,86 **A**	73,28 **B**	84,53 **AB**	
LSD Values	LSD cultivar= 12.06* LSD NaCl treat. = 13.93 LSD cult. x NaCl treat. = 24.13				

*Different letters among cultivars and NaCl treatments denote significant differences (LSD test, $p<0.05$)

Regarding hypocotyl lengths of cultivars/types, radish cultivars differed significantly in hypocotyl length after NaCl treatment. The highest hypocotyl length was found in radish Antep under the highest NaCl concentration whereas the control was had the lowest highest hypocotyl length (Table 3). Hypocotyl lengths differed greatly based on NaCl concentration levels. Similar results in cultivar differences in hypocotyl length were also reported by Zaman et al., 1995 and Gosh et al., 2014.

Table 3. Effect of NaCl concentrations on hypocotyl lengths

Hypocotyl Length (mm)					
	NaCl Concentrations				Means of cultivars/types
Cultivar	Control	100 mg L^{-1} NaCl	200 mg L^{-1} NaCl	300 mg L^{-1} NaCl	
Radish Antep	36,66 d	63,49 abc	60,24 bc	82,60 a	60,75
Fındık Radish	51,22 cd	67,10 abc	51,49 cd	64,94 abc	58,69
Black Radish	61,66 bc	72,49 ab	58,94 bc	69,27 abc	65,59
Means of NaCl treatments	49,84 **C**	67,70 **AB**	56,89 **BC**	72,27 **A**	
LSD Values	LSD cultivar= 9.58 LSD NaCl treat. = 11.07* LSD cult. x NaCl treat. = 19.26				

*Different letters among cultivars and NaCl treatments denote significant differences (LSD test, $p<0.05$)

CONCLUSIONS

In the current study, three different radish cultivars/types germination features in different NaCl concentrations were evaluated. Increasing the salt concentration impacted the germination percentage of radish cultivars/types differently. The black radish cultivar/type showed better tolerance to different NaCl concentrations. Root and hypocotyl lengths differed greatly based on NaCl concentration levels regarding cultivars/types, also. Fındık and black radishes performed better than Antep radish in terms of root lengths, while Antep radish showed the longest hypocotyl length. Based on the findings of the current study, different NaCl concentrations can be recommended to improve the tolerance of cultivated plants under conditions of increased soil salinity.

REFERENCES

Ahmad, P., A. Kumar, M. Ashraf, N.A. Akram, 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). African Journal of Biotechnology 11(11): 2694-2703. doi:10.5897/AJB11.3203

Ahmad, P., S. Sharma. 2012. Physio-biochemical attributes in two cultivars of mulberry (*Morus alba* L.) under $NaHCO_3$ stress. International Journal of Plant Production 4(2): 79-86. doi: 10.22069/IJPP.2012.685

Coopland, O.L., M.B. McDonald. 1995. Seed Science and Technology. 3rd edition, Chapman and Hall., New York, pp: 240.

Cuartero, J., R. Fernandez–Munoz. 1999. Tomato and salinity. Scienta Horticulturae. 78: 83–125.

Ghosh, P., P. Kumar Dash, R. Sarker, A. Mannan. 2014. Effect of salinity on germination, growth and yield of radish (*Raphanus sativus* L.) varieties. International Journal of Biosciences 5(1): 37-48. doi: 10.12692/ijb/5.1.37-48

Kanjevac, M.M., B.M. Bojović, M.S. Todorović, M.S. Stanković. 2021. Effect of Seed Halopriming On Improving Salt Tolerance In *Raphanus sativus* L. Kragujevac J. Sci., 43: 87-98.

Khan, M.A, I.A. Ungar, A.M. Showalter. 2000. Effect of sodium chloride treatments on growth and ion accumulation of the halophyte *Haloxylon recurvum*. Commun. Soil. Sci. Plant Anal. 31(17–18): 2763–2774.

Lu, Z.L., L.W. Liu, X.Y. Li, Y.Q. Gong, X.L. Hou, X.W. Zhu, L.Z. Wang. 2008. Analysis and evaluation of nutritional quality in Chinese radish (*Raphanus sativus* L.). Agricultural Sciences in China 7(7): 823-830.

Murillo–Amador, B., R. Lopez–Aguilar, C. Kaya, J. Larrinaga–Mayoral, A. Flores–Hernandez. 2002. Comparative affect of NaCl and polyethylene glycol on germination emergence and seedling growth of cowpea. J. Agronomy and Crop Science. 188: 235–247.

Othman, Y. 2005. Evaluation of Barley Cultivars Grown in Jordan for Salt Tolerance. Ph.D Thesis, Jordan University of Science and Technology, Jordan.

Rahman, M., U.A. Soomro, M. Zahoor-ul-Haq and S. Gul. 2008. Effects of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars. World Journal of Agricultural Sciences. 4(3): 398–403.

Vishnu Priya, S., R. Elavarasan, T. Selvan Kumar. 2020. Studies on radish salinity tolerance and their growth response analysis. International Journal of Advanced Research in Biological Sciences 7(5): 30-39. doi: 10.22192/ijarbs.2020.07.05.004

Wang, Y., Y. Pan, Z. Liu, X. Zhu, L. Zhai, L. Xu, L. Liu. 2013. De novo transcriptome sequencing of radish (*Raphanus sativus* L.) and analysis of major genes involved in glucosinolate metabolism. BMC genomics 14(1): 1-13. doi: 10.1186/1471-2164-14-836.

Zaman, S.K, D.A.M. Choudhury, N.I. Bhuiyan. 1995. Effect of salinity on germination, growth, yield and mineral composition of rice. Bangladesh Journal of Agricultural Science 24: 103-109.

THE DIVERSITY OF FAUNA OF THE ORTHOPTERA ORDER IN THE VLORA AREA

Gerta Subashaj [*1]*; Eltjon Halimi* [2]*; Anila Paparisto* [3]

[1]*Reald University College, Faculty of Medical Technical Sciences, Vlora, Albania*

[2,3] *University of Tirana, Faculty of Natural Sciences, Tirana, Albania*

Corresponding author e-mail: gerta.subashaj@fshnstudent.info

https://orcid.org/0009-0000-2112-4297

ABSTRACT

Orthoptera is an order of insects that may be found in several habitats. Additionally, they play a crucial function in the food chain. This study aimed to ascertain the taxonomic classification of species within various habitats in the Vlora geographical region over the period of June to September 2023. This study pertains to 18 species belonging to the order Orthoptera and 4 different families. The Tettigoniidae family has the greatest species variety, consisting of a total of 9 distinct species. At the same time, there is just one species that represents each of the families *Tetrigidae* and *Mogoplistidae*. The Sevaster station is represented by ten species, which is the most number. This station provides the most favorable circumstances 4 species belonging to the order Orthoptera.

Keywords: Orthoptera, Diversity, Grasshoppers, Ecosystem, Vlora

INTRODUCTION

State that the order Orthoptera, classified under the class Insecta, is among the most ancient assemblages of insects (Matthew et al. 2022). Have together established that grasshoppers are the most diverse and largest group of insects, capable of thriving in many conditions (Zahri et al. 2023; Bhowmik et al. 1982; Sageer et al. 2023). (Gardiner, 2018) asserts that orthoptera play a vital role in the functioning of the food chain. Have categorized them into two distinct categories: Caelifera, which refers to short-antennae shrimps, and Ensifera, which refers to long-antennae shrimps (Panhwar, 2015; Ali et al. 2017). According to (Gupta et al. 2016), both filiform and ensiform antennae are feasible. Have all recorded instances of large-scale outbreaks of species classified under the Order Orthoptera (Uvarov, 1966; Uvarov, 1977; Kasalo et al. 2024). This phenomena is widely recognized from both a biological and economic standpoint. (Kasalo et al. 2024) found that certain species of the Acrididae family (Caelifera) display seasonal polymorphism, or polyphenism, in several aspects such as their physical characteristics, growth, chemical makeup, and interaction with the environment.

Research undertaken by (Barsyte, 1999; Warchalowska-Sliwa et al. 2005; Guria, 2024) has shown that Orthoptera species may be used to assess the detrimental impacts of different environmental pollutants.

MATERIALS DHE METHODS

From June to September 2023, the species were collected in the Vlora area at three stations: Sevaster, Gumenicë, and Shesh (Vranisht).

Aerial ethnological nets (NETs) were used to collect species of the Order Orthoptera in different ecosystems. The collected species were placed in tubes with etheric acid, and each tube was associated with the corresponding label (Colas, 2000). In these tubes, they were transported to the laboratory. They were observed with a stereomicroscope (Prefix Sciences) for the taxonomic identification of the collected species based on external characteristics, and the keys and literature from the countries bordering Albania, which have habitats, were used like the Study Area (Willemse et al., 2018; Lemonnier–Darcemont et al., 2018) as well as previous publications on the Orthopeda Order of Albania (Csiki, 1922; Subashaj et al., 2024).

RESULTS AND DISCUSSIONS

In the present investigation, we make reference to the list of species belonging to the Order Orthoptera that is displayed in Table 1. This list is arranged in accordance with the stations Sevaster, Gumenicë, and Shesh (Vranisht).

Table 1: List of species for the Acrididae family

Nr	Scientific name	Sevaster	Gumenica	Shesh
1	**Family *Tettigoniidae*** Krauss, 1902			
1	*Decticus albifrons* Fabricius, 1775	+		
2	*Poecilimon jonicus F*ieber, 1853			+
3	*Acrometopa servillea macropoda* Burmeister, 1838			+
4	*Eupholidoptera schmidti* Fieber, 1861	+		+
5	*Tettigonia viridissima* Linnaeus, 1758			+
6	*Decticus verrucivorus* Linnaeus, 1758	+		
7	*Pholidoptera griseoaptera* De Geer, 1773	+		
8	*Pholidoptera femorata* Fieber, 1853	+		
9	*Pholidoptera aptera* Fabricius, 1793	+		
2	**Family *Acrididae*** MacLeay, 1819			
10	*Acrida ungarica mediterranea* Herbst, 1786	+		
11	*Acrotylus patruelis* Herrich-Schäffer, 1838		+	
12	*Calliptamus italicus* Linnaeus, 1758	+		
13	*Oedipoda caerulescens* Linnaeus, 1758	+	+	
14	*Chorthippus bornhalmi* Linnaeus, 1758			+
15	*Chorthippus biguttulus* Linnaeus, 1758			+
16	*Oedaleus decorus* Germar, 1825	+		
3	**Family *Tetrigidae*** Rambur, 1838			
17	*Paratettix meridionalis* Rambur, 1838		+	
4	**Family *Mogoplistidae*** Brunner von Wattenwyl, 1873			
18	*Arachnocephalus vestitus* Costa, 1855		+	

According to the taxonomic analysis of the Order Orthoptera, we were able to identify 18 species and 4 families in this study. These findings were based on the stations stated above, which were located in a variety of environments (Table 2).

Table 2 Distribution of the number of species and species frequency according to familie

Nr	**Family**	**Species' number**	**Species frequency**
1	*Tettigoniidae*	9	50 %
2	*Acrididae*	7	38.88 %
3	*Tetrigidae*	1	5.55 %
4	*Mogoplistidae*	1	5.55 %

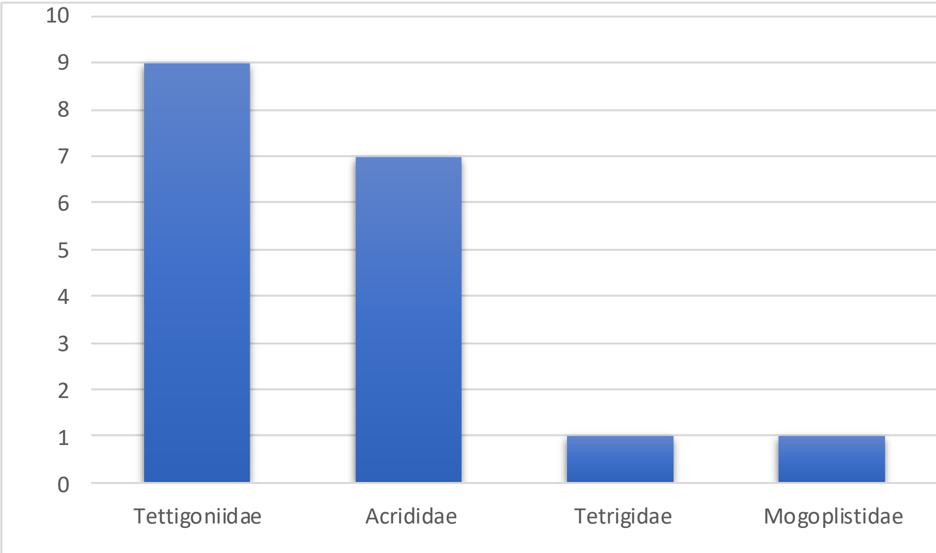


Figure 1 Distribution of the number of species by families

Upon examining the data shown in Table 2 and Figure 1, it is evident that the *Tettigoniidae* family has the highest level of species diversity. This family has a total of 9 species and has a species frequency of 50 %. The *Acrididae* family, with 7 distinct species, has a species frequency of 38.88 %. As previously mentioned, the *Tettigoniidae* and *Acrididae* species play a crucial role in creating optimal conditions within ecosystems. Conversely, the *Tetrigidae* and *Mogoplistidae* families each consist of only 1 species, and the frequency of these species within their respective families is 5.55 %.

Table 3 Number of species by station

Station	**Species number**	**species frequency**
Sevaster	10	55.55 %
Gumenicë	4	22.22 %
Shesh (Vranisht)	6	33.33 %

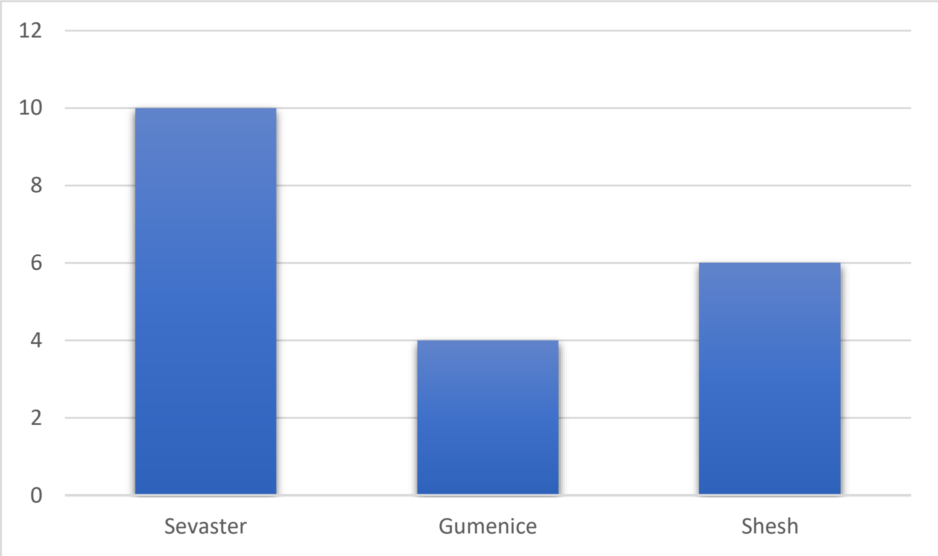


Figure 2 Distribution of species according to stations

The examination of habitat diversity in the examined region, as reported in Table 3 and Figure 2, shows that the Sevaster station is home to 10 out of the total number of species, representing a frequency of 55.55 %. Next in line is the station of Shesh (Vranisht), which is home to 6 different species. The species frequency at this station is 33.33 %. Following Shesh is the station of Gumenica, which has 4 species and a species frequency of 22.2 %. Interestingly, the species belonging to the Order Orthoptera are least likely to choose Gumenica as their habitat due to human activities in the area. During this time, the stations Sevaster and Shesh offer optimal conditions for the species belonging to the Order Orthoptera.

CONCLUSIONS

This research offers a thorough examination of the species belonging to the Order Orthoptera that inhabit the various habitats of the Vlora area. It provides significant information regarding their classification and geographical distribution. Our investigation indicates that there are 18 different species and 4 families within the Order Orthoptera in the Vlora area. This demonstrates the intricate and diverse nature of the ecosystems in this region.

The family *Tettigoniidae* has the highest number of species, with 9, followed by the family *Acrididae,* which has 7 species, making them the most diverse families within the Order Orthoptera. Both the families *Tettigoniidae* and *Mogoplistidae* consist of only one species apiece. The Tettigoniidae family, known for its extensive variety of species, has a substantial impact on this study.

The Sevaster station, which has 10 species from the whole spectrum of species variety, plays a significant role in this investigation. Based on the study's findings, this station provides the most advantageous environment for species belonging to the Order Orthoptera in comparison to the other stations.

ACKNOWLEDGMENTS

The authors want to thank their French colleagues, *M. Darcemont-Lemonnier* and *Ch. Darcemont*, for their invaluable assistance in preparing this publication. The University of Tirana also supported this project through the UT-Search, Excellence, and Innovation—REI2023 program.

REFERENCES

Ali, S., Panhwar WA, Riffat S, Mehmood SA, Wagan MS. *First record of the genus Mermiria Stål with its species Mermiria bivittata (Serville, 1838) from Pakistan (Orthoptera, Acrididae, Gomphocerinae)*. Journal of Entomology and Zoology Studies. 2017. 5(1):279-281.

Ali, Sh., Panhwar, A., W. *A checklist of acrididae (Orthoptera) of Hazara Division Khyber Pakhtunkhwa Pakistan*. Journal of Entomology and Zoology Studies. 2017. 5(5): 96-100.

Barsyte, D. *The analysis of genotoxic damage of industrial pollution infreshwater mollusc tissues.* Summary Doctoral Thesis, Institute of Ecology.1990. Vilnius. 1–20.

Bhowmik, H., K., Rui, K., N. *Notes on a collection of grasshoppers (Orthoptera: Acrididae) from Siwalik hills*. Indian Museum Bulletin. 1982. 17: 48–54.

Colas, G. *Guide de l'entomologiste*. Editions N. Boubée. 2000.

Csiki, E. *Explorationes zoologicae ab C. Csiki in Albania peractae. VI. Orthopteren*. Magyar Tudomanyos Akademia. 1922. 1, 75-82.

Gardiner, T. *Grazing and Orthoptera: a review*. Journal of Orthoptera Research. 2018. 27(1): 3–11.

Gupta, K., S., Chandra, K. *Diversity of Orthoptera (Insecta) fauna of Achanakmar Wildlife Sanctuary, Bilaspur, Chhattisgarh, India*. Journal of Asia-Pacific Biodiversity. 2016. 1–13.

Guria, S., Ali, R. *Study of Hemocytes of Grasshoppers collected from the Surrounding areas of Jaldapara forest and Rajabhatkhawa in comparison with other areas*. Research and Reviews in Microbiology Volume I. 2014.

Kasalo, N., Tvrtković, N., Bogić, D., Kokan, B., Vuković, M., Kučinić, M., Skejo, J. *An Overview of Orthoptera Mass Occurrences in Croatia from 1900 to 2023*. 2024. *15*(2), 82.

Lemonnier – Darcemont, M., Kati, V., Willemse, L., Darcemont, CH. *Effects of changing grazing systems on the threatened genus Peripodisma (Orthoptera: Acrididae: Melanoplinae) in the Mediterranean mountains of the southern Balkans*. Journal of Mediterranean Ecology vol. 16, 2018: 67-79.

Mathew, R., Leema, R., Joseph, K., G., Cyril, A., E. *Orthopteran diversity in Tropical ecosystems of Central Kerala, India*. Indonesian Journal of Forestry Research. 2022. Vol. 9 No. 1, 121-133.

Panhwar, W., A. *Studies on the Systematic and Ecological Status of Tettigonioidea (Ensifera) of Pakistan*. PhD Thesis Submitted To Department of Zoology, University of Sindh, 2015, 1–242.

Sageer, E., Th., Chenthamarakshan, B., Bhaskar, Dh. *Grasshoppers, crickets and katydids of Kerala, an updated checklist for the order Orthoptera*. 2023. ENTOMON 48(3): 331- 356.

Subashaj, G., Halimi, E., Paparisto, A. *An Overview of the Grasshoppers (Orthoptera, Insecta) in Vlore Area, Albania*. International Journal of Advanced Multidisciplinary Research and Studies (Int. j. adv. multidisc. res. stud.). 2024. 4(3):316-319.

Uvarov, B.P. *Grasshoppers and Locusts.* A Handbook of General Acridology. Vol. 1. Anatomy, Physiology, Development, Phase Polymorphism, Introduction to Taxonomy; Cambridge University Press: Cambridge, UK, 1966; pp. 1–481.

Uvarov, B.P. *Grasshoppers and Locusts.* A Handbook of General Acridology Vol. 2. Behaviour, Ecology, Biogeography, Population Dynamics; Centre for Overseas Pest Research: London, UK, 1977; pp. 1–622.

Zahri, A., Radouane, N., Ezrari, S., Errabhi, N. *Review of the Taxonomic Status of the Most Frequent Orthoptera Species (Caelifera: Acrididae) in the Middle Atlas of Morocco*. Indian Journal of Entomology. 2023.

Warchalowska–Sliwa E, Niklinska M, Görlich A, Michailova P and Pyza E. *Heavy metal accumulation, heat shock protein expression and cytogenetic changes inTetrix tenuicornis (L.) (Tetrigidae, Orthoptera) from polluted areas*. Environmental Pollution, 2005. 133(2). 373-381.

Willemse, L., Kleukers, R., Odé, B. The grasshoppers of Greece. EIS Kenniscentrum Insecten & Naturalis Biodiversity Center, Leiden. Naturalis Biodiversity Center. 2018. 440pp.

AN OVERVIEW OF THE ACRIDIDAE FAMILY (ENSIFERA - ORTHOPTERA) IN THE VLORA REGION, SOUTH-WESTERN ALBANIA

Gerta Subashaj[1]; Eltjon Halimi[2]; Dritan Topi[3], Anila Paparisto[4]*

[1]*Reald University College, Faculty of Medical Technical Sciences, Vlora, Albania*

[2, 3,4]*University of Tirana, Faculty of Natural Sciences, Tirana, Albania*

Corresponding author e-mail: gerta.subashaj@fshnstudent.info

https://orcid.org/0009-0000-2112-4297

ABSTRACT

This study intends to provide a comprehensive account of the species of Short-Horned Grasshoppers belonging to the family Acrididae (Orthoptera) that may be discovered in the various habitats of the Vlora region, which is located in the southern part of Albania. A well-defined seasonal pattern that includes a cold and rainy winter and a warm and arid summer is characteristic of the climate in this region, which shows some characteristics that are typical of the Mediterranean climate. On a worldwide scale, this region represents a discovery that is both extraordinarily valuable and one of a kind. Following an examination of the data, it has been determined that the genera *Calliptamus*, *Chorthippus*, and *Oedipoda* are known to exist, with two species belonging to each of these genera. It is estimated that 13.33% of each species is present. For the purpose of establishing whether or not these species are present in this region, the Llogara and Armen National Parks were both investigated. According to the conclusions of the study, these parks, which each include six species and have a species frequency of forty percent, offer the best possible environment for the species in question.

Keywords: Orthoptera, Acrididae, Habitat, Biodiversity, Vlora, Southwestern Albania

INTRODUCTION

The Acrididae family, is a diversified group within the Orthoptera order. These grasshoppers are generally referred to as short-horned grasshoppers (Memon et al. 2021). According to (Riffat, 2015; Shaikh et al. 2018), the agricultural crops that they infest are considered to be pests if they occur. In addition to wetland environments, they may be discovered in a wide variety of habitats, including as grasslands, shrublands, herbaceous regions, and woods. (Dempster, 1963; Uvarov, 1966; Shaikh et al. 2018) all came to the conclusion that the majority of the species that belong to the Acrididae family are herbivorous insects.

The findings of the study conducted by (Akwanjoh, 2020) suggest that they have the potential to function as helpful bio-indicators for identifying changes in the environment. According to (Song et al. 2018), the family Acrididae is comprised of a wide variety of species that fall within the order Orthoptera and the suborder Caelifera.

Each and every member of the Acrididea family contains a pair of antennae that are extremely small and very thin. In contrast to the tympanum, which is situated on the lateral sides of the first abdominal segment, the tarsi are composed of three segments. The research conducted by (Smith et al. 2014) revealed that certain adult species have wings, whereas others either do not have wings at all or have wings that are severely reduced. In this paper, we provide the results of our taxonomic investigation into the species belonging to the Acrididae family that were found in the Vlora region.

MATERIALS AND METHODS

During the years 2022 and 2023, the species were gathered from a variety of locations within the Vlora region, including National Park Llogara, Armen, and Selenica. The different species were gathered by the utilization of aerial entomological nets (NET). In order to maintain the color, the species were placed in tubes that contained ether (Colas, 2000; Halimi et al., 2023). Each tube was then labeled with the appropriate information, which included the date, location, coordinates, and altitude from the data collection. Notes were recorded in a journal regarding the vegetation, as well as any particular traits that were considered to be unique to particular species. For the taxonomic identification, the species were observed with a stereomicroscope (Perfex Sciences), and the keys from the publications for the countries bordering Albania, which offer similar climatic conditions and habitats to our study area, were used (Eades et al., 2014; Willemse et al. al., 2018; Willemes, 1985) as well as previous publications on the Order Orthopetra of Albania (Csiki, 1922; Ebner, 1910; Lemonnier – Darcemont & Darcemont, 2015; Subashaj, 2024).

RESULTS AND DISCUSSIONS

This study presents the species of the Acrididae family, which are listed in Table 1 according to the stations (Llogara, Armen, and Sevaster National Parks) in the habitats of the Vlora area, which is situated in the southwestern part of Albania, across the Ionian Sea, and in the southern Adriatic Sea. The Vlora area is located in the southern Adriatic Sea.

Table 1. List of species of the Acrididae family

No.	Specie	Llogara National Park	Armen	Sevaster
1	**Genus Chorthippus**			
1	*Chorthippus mollis mollis* Charpentier, 1825	+		
2	*Chorthippus bornhalmi* Harz, 1971	+		
2	**Genus Oedipoda**			
3	*Oedipoda caerulescens* Linnaeus, 1758	+	+	+
4	*Oedipoda germanica* Latreille, 1804	+		
3	**Genus Pezotetix**			
5	*Pezotettix giornae* Rossi, 1794	+		
4	**Genus Euchorthippus**			
6	*Euchorthippus declivus* Brisout, 1848	+		
5	**Genus Sphingonotus**			
7	*Sphingonotus caerulans* Linnaeus, 1767		+	
6	**Genus Pseudochorthippus**			
8	*Pseudochorthippus parallelus tenuis* Zetterstedt, 1821		+	
7	**Genus Paracaloptenus**			
9	*Paracaloptenus caloptenoides* Brunner von Wattenwyl, 1861		+	
8	**Genus Acrotylus**			
10	*Acrotylus patruelis* Herrich-Schaffer, 1838		+	
9	**Genus Acryptera**			
11	*Arcyptera sp* Serville, 1839		+	
10	**Genus Calliptamus**			
12	*Calliptamus sp* Serville, 1831			+
13	*Calliptamus italicus* Linnaeus, 1758			+
11	**Genus Oedaleus**			
14	*Oedaleus decorus* Germar, 1825			+
12	**Genus Acrida**			
15	*Acrida ungarica mediterranea* Herbest, 1786			+

Twelve genera and fifteen species are included in the collection of species belonging to the Acrididae family that was collected from three different sites (Table 2, Figure 1).

Table 2. Species and genus enlisted

Genus	Species number	Species frequency
Acrida	1	6.66%
Acrotylus	1	6.66%
Acryptera	1	6.66%
Calliptamus	2	13.33
Chorthippus	2	13.33
Euchorthippus	1	6.66%
Oedaleus	1	6.66%
Oedipoda	2	13.33
Paracaloptenus	1	6.66%
Pezotetix	1	6.66%
Pseudochorthippus	1	6.66%
Sphingonotus	1	6.66%

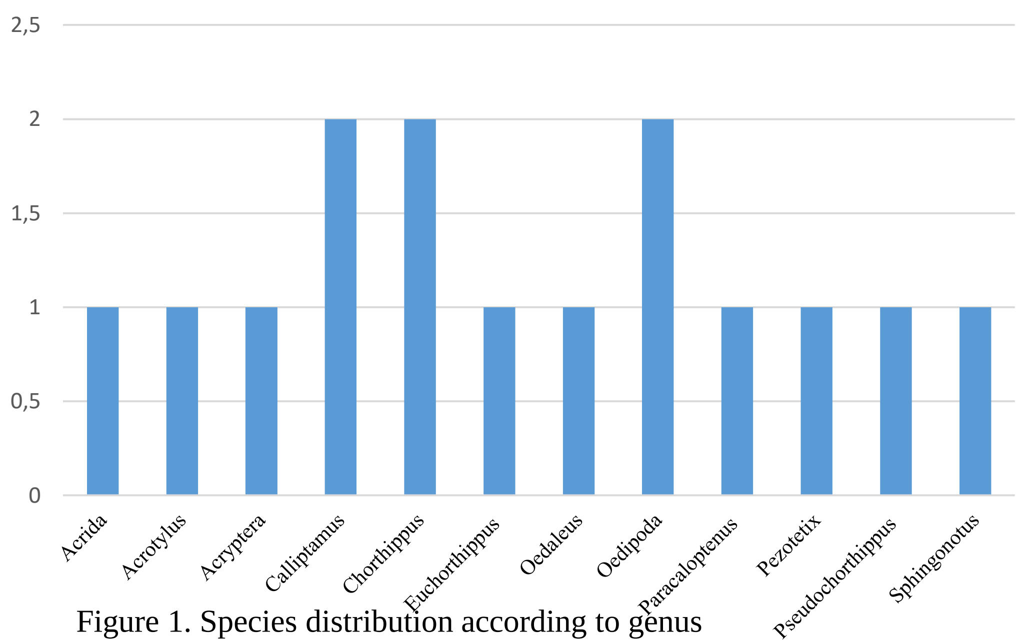


Figure 1. Species distribution according to genus

Based on the data, it can be deduced that the genera *Calliptamus*, *Chorthippus*, and *Oedipoda* are responsible for two species out of the total number of species. Thirteen point three percent of the total number of species in the entire collection is accounted for by these genera. The genera *Acrida*, *Acrotylus*, *Acryptera*, *Euchorthippus*, *Oedaleus*, *Paracaloptenus*, *Pezotetix*, *Pseudochorthippus*, and *Sphingonotus* are the ones that come in close succession after them. Due to the fact that each of these genera has one species, the total number of species that may be found in each of these genera is one. The frequency of each species within each genus is found to be six point six percent. According to the findings of the inquiry into the diversity of stations, which are shown in table 3 and figure 2, the Llogara and Armen National Park stations each include six species, which accounts for forty percent of the total number of species. These findings are presented in the table and figure respectively. Each of the five unique species that make the Sevasteri station their home has a frequency of 33.33 percent, and the station is home to all five of these species. The stations in Llogara and Armen National Park offer the most optimal environments for species that are members of the Acrididae family. As a consequence, the amount of disturbance that is generated by human activities is reduced to the greatest extent possible.

Table 3. Species according to the surveyed stations

Station	**Species number**	**Species frequency**
Parku Kombetar Llogara	6	40 %
Armen	6	40 %
Sevaster	5	33.33 %

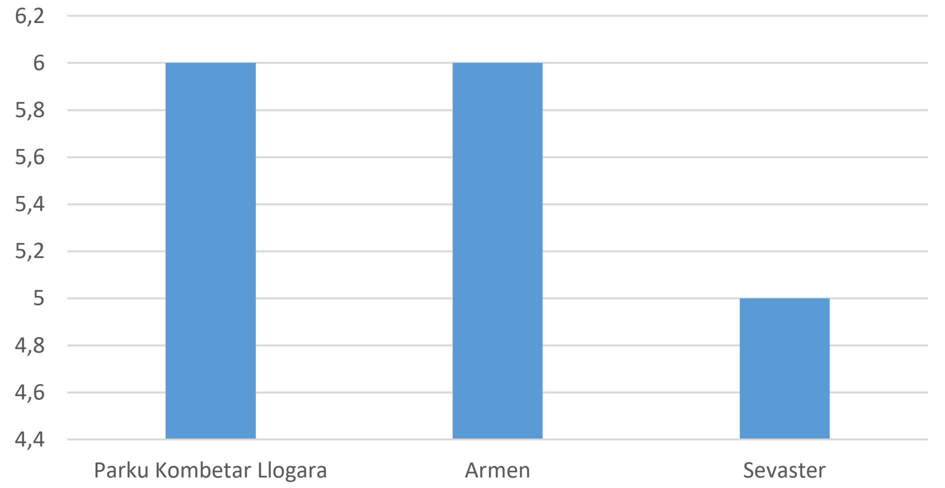


Figure 2. Species distribution according to studied

CONCLUSIONS

This research presents a complete analysis of the species that belong to the Acrididae family and are found in the ecosystems of the Vlora region. These species are found in the Vlora region. According to the parameters of this inquiry, we are discussing a total of twelve different genera and fifteen different species. A great degree of species diversity can be found within the Acrididae family, notably among the genera *Calliptamus*, *Chorthippus*, and *Oedipoda*, each of which has two different species. Acrididae is a family of insects. These three genera collectively are responsible for thirteen-point three percent of the total number of species that have ever been discovered. With a total of six species, the stations in Llogara and Armen National Park have the most species diversity of all of the stations in the park. This represents forty percent of the total species diversity count.

According to the information shown here, it is possible to draw the conclusion that these stations provide the conditions that are most favorable for the species that belong to the Acrididae family.

ACKNOWLEDGMENTS

The authors wish to thank their French colleagues, M. Darcemont-Lemonnier and Ch. Darcemont, for their invaluable assistance in preparing this publication. Additionally, the University of Tirana provided support for this project through the "*UT-Search, Excellence, and Innovation—REI2023*" program.

REFERENCES

Akawanjoh, R., S., Tita, M., Nj. First Ever Data on the Occurrences of Acrididae (Acridoidea: Orthoptera) Grasshoppers in the North West Region of Cameroon. Journal of Biology and Life Science. 2020. 11/1.

Colas, G. Guide de l'entomologiste. Editions N. Boubée. 2000.

Csiki, E. Explorationes zoologicae ab C. Csiki in Albania peractae. VI. Orthopteren. Magyar Tudomanyos Akademia. 1922. 1, 75-82.

Dempster, JP. The population dynamic of Grasshoppers and Locust Reprinted from the Biological Review. 1963. 38:491-493.

Eades, D.C., Otte, M., Cigliano, M.M., Braun, H. Orthoptera Species File. 2014. Version 5.0/5.0.

Esler, K.J., A L. Jacobsen, and R. B. Pratt, 'Characteristics of Mediterranean-Type Ecosystems', The Biology of Mediterranean-Type Ecosystems, Biology of Habitats Series (Oxford, 2018; online edn, Oxford Academic), doi.10.1093/oso/9780198739135.003.0002.

Halimi, E., Paparisto, A., Alameti, E. Taxonomic and ecological data on the Hemiptera fauna from Berati region habitats in Albania. Journal of Biological. Research–Bollettino della Società Italiana di Biologia Sperimentale, 2023. 96(1).

Ebner, R. Ein Beitrag zur Orthopterenfauna der europäischen Türkei mit besonderer Berücksichtigung von Albanien (Ergebnisse einer Reise nach Nord-Albanien, A. Klaptocz, 1909). Zoologische Jahrbücher, Abteilung für Systematik, Ökologie und Geographie der Tiere. 1910. 29, 401-414.

Lemonnier – Daecemont, M., Darcemont, Ch. Peripodisma ceraunii (Orthoptera, Acrididae, Melanoplinae: Podismini), a new species of the genus Peripodisma from southern Albania. Articulata. 2015. 30, 53–62.

Memon, R., P., Panhwar, A. W. Study on some selected species of Acrididae (Orthoptera) from district Dadu, Sindh-Pakistan. Journal of Entomology and Zoology Studies. 2021. 9(1), 06-11.

Riffat, S. Wagan, M., S. Grasshoppers and locusts of Pakistan, Higher Education Commission, Pakistan. 2015. 1-180. ISBN: 978-969-417-180-7.

Smith, R., T., Froeba, G., J., Capinera, J. Key to the GRASSHOPPERS (Orthoptera: Acrididae) Of Florida. 2009. Florida Entomologist 87. 2004. 537-550. DOI:10.1653/00154040(2004)087[0537:KTTGOA]2.0.CO;2.

Song, H., Pérez, M., Woller., A., Cigliano., M. Evolution, Diversification, and Biogeography of Grasshoppers (Orthoptera: Acrididae). Insect Systematics and Diversity. 2018. 2(4): 3; 1–25.

Subashaj, G., Halimi, E., Paparisto, A. An Oreview of the Grasshoppers (Orthoptera, Insecta) in Vlore Area, Albania. International Journal of Advanced Multidisciplinary Research and Studies (Int. j. adv. multidisc. res. stud.). 2024. 4(3):316-319.

Shaikh, N., Sultana, R. Comparative study on the morphometric characteristics of egg-pods in various subfamilies of Acrididae. Journal of Entomology and Zoology Studies. 2018. 6(1), 1423-1426.

Uvarov, BP. Grasshoppers and Locust: A handbook of General Acridology, 1966.

Willemes, F. A key to the orthoptera species of Greece. Volume 2 Fauna Graeciae. Hellenic Zoological Society Athens. 1985. 288pp.

Willemse, L., Kleukers, R., Odé, B. The grasshoppers of Greece. EIS Kenniscentrum Insecten & Naturalis Biodiversity Center, Leiden. Naturalis Biodiversity Center. 2018. 440pp.

NEW OIL-YIELDING BULGARIAN SUNFLOWER HYBRID "KRASI"CLP" (HELIANTHUS ANNUUS L.)

Georgi Georgiev, Nina Nenova, Galin Georgiev

Dobrudzha Agricultural Institute - city G. Toshevo
Corresponding author: Georgi Georgiev, E-mail: georgi_d4@abv.bg

ABSTRACT

Hybrid Krasi CLP is a new Bulgarian two-line sunflower hybrid suitable for cultivation according to Clearfield Plus technology, obtained by inter-line hybridization. The hybridization involved the maternal sterile line 1111A as a component and the fertility restorer line GTS 51R. The two parental lines have very good general and specific combinatorial ability. Hybrid Krasi is medium-early with a vegetation period of 109-111 days, seed diameter 19-21cm, mass of 1000 seeds 62g, kernel content %-77%. The production potential, depending on the conditions of the year, varies from 328 kg to 350 kg. The oil is of the linoleic type and its content varies from 49-51%. The hybrid is characterized by good drought tolerance, resistance to downy mildew 731, phoma, phomopsis, alternaria and blue wrist. The hybrid was recognized by the Minister of Agriculture of the Republic of Bulgaria under the RHS with order RD-12-3/17.03.2023. The hybrid was tested at IASAS for two years in three locations.

Keywords: Sunflower, Drought tolerance, Hybrid, Yield traits, Yield performance,

INTRODUCTION

Seed yield in sunflower is a quantitatively heritable component strongly influenced by environmental factors. However, the yield also depends on the genetic potential of the hybrids and on other components related to seed yield such as seed mass, kernel diameter, mass per 1000 seeds, leaf area, plant height. The yield of the oil-bearing sunflower is also determined by the agrometeorological conditions of the periods of the individual phenophases: sprouting-budding, sprouting-mass flowering, flowering period and vegetation period. Sunflower breeders should pay attention to these important seed yield components when evaluating experimental hybrids. A major factor determining the achievement of a high and quality sunflower yield is the selection of hybrids suitable for the area, providing high seed yield, combined with high oil content, resistance or tolerance to the most important diseases, enemies and parasites (Georgiev et al., 2012; Georgiev, 2016). It is very difficult and almost impossible to collect all these indicators in a single hybrid.

The aim of the present study is to make a complete characterization of their newly created tolerant Bulgarian hybrid Krasi CLP, created at the Dobrudzhan Agricultural Institute - General Toshevo. Hybrida is recognized by the Minister of Agriculture of the Republic of Bulgaria under RHS with order RD-12-3/17.03.2023. The hybrid was tested at IASAS for two years in three locations.

In recent years, high-yielding parental lines and hybrids with a complex of valuable quantitative and qualitative indicators have been created at the Dobrudzhan Agricultural

Institute - G. Toshevo. The newly created sunflower hybrids showed very good stability to abiotic and biotic environmental factors. With these sunflower hybrids, very good results of seed yield kg/ha were obtained in different regions of Europe and in no way inferior to the hybrids from other countries.
In sunflower, seed yield and oil content are complex characteristics that are influenced by different factors that may act individually or collectively. The study of a complex of traits is a crucial approach to increase seed yield. (Chandirakala et al., 2015) [5]. The effectiveness of selection depends mainly on the direction and magnitude of the relationship between yields and its components. Correlation describes the mutual relationship between variables and helps to improve different features simultaneously. An attempt was therefore made to estimate a correlation between yield and yield components. We have and maintain the largest collection of wild sunflower species on the Balkan Peninsula, including annual and perennial forms (Valkova et al., 2015; Valkova et al., 2016).

MATERIAL AND METHODS

Hybrid Krasi CLP is a new Bulgarian two-line sunflower hybrid suitable for cultivation according to Clearfield Plus technology, obtained by inter-line hybridization. The hybridization involved the maternal sterile line 1111A as a component and the fertility restorer line GTS 51R. The two parental lines have very good general and specific combinatorial ability. Hybrid Krasi is medium-early with a vegetation period of 109-111 days, seed diameter 19-21cm, mass of 1000 seeds 62g, kernel content %-77%. The production potential, depending on the conditions of the year, varies from 328 kg to 350 kg. The oil is of the linoleic type and its content varies from 49-51%. The hybrid is characterized by good drought tolerance, resistance to downy mildew 731, phoma, phomopsis, alternaria and blue wrist. The hybrid was recognized by the Minister of Agriculture of the Republic of Bulgaria under the RHS with order RD-12-3/17.03.2023. The hybrid was tested at IASAS for two years in three locations.

RESULTS AND DISCUSSION

Biological and economic qualities of hybrid Krasi CLP
The Krasi CLP hybrid has been tested three years in a row in a competitive varietal experiment on the fields of the DZI. It showed an excess compared to the average standard in terms of seed yield and oil yield per hectare (Table 1).

Table 1. Results of Krasi CLP hybrid testing in KSO - 2020, 2021.

Seed yiled kg/da	**% of Average Standard % of average standard**	**Oil content %**	**Oil yield кg/da**	**% of Average Standard % of average standard**
2020				
359,6	103.7	48.1	159.3	106.8
2021				
337,8	104.2	47.6	168.8	107.7

After a two-year trial in KSO, the Krasi CLP hybrid was included in the Single Variety Trial in DZI in 2018. The results showed that this year the seed yield was 368.7 kg/da, which was 6.6% higher than the average standard, and the content of oil in the seeds is 51.7%. The amount of oil per hectare is 176.5 kg/da or 109% exceeding the standard.
Hybrid Krasi CLP was tested for biological and economic qualities in the experimental fields of IASAS for two years - 2020 and 2021. On average for the period of the test, 336 kg/da of seeds were obtained on average for the two years of the test.

Table 2. Results of the Krasi CLP hybrid test in IASAS - 2020, 2021.

№	Hybrid	Year of testing	Selanovci			Brashlen			Radnevo			Average	
			kg/da		%	kg/da		%	kg/da		%	kg/da	%
3	**Krasi CLP**	**2**	359	-	100,0	339	-	100,5	287	oo	88,3	**328**	96,4

Results of a varietal test for biological and economic qualities of the Krasi CLP hybrid

Table 3. Results of a varietal test for biological and economic qualities of the Krasi CLP hybrid

Hybrid / KRASI CLP	**PH**	**HD**	**LP**	**BRL**	**MS1P**	**M1000S**	**NS1P**	**VEGP**	**PBUT**	**PNC**	**PCAF**
	164	21	7830	28	114,6	60,5	2445,6	110	38	70	13

Legend: PH-Height of plant cm, HD-Diameter of pita cm, LP-Leaf area cm2 ,BRL-Number of leaves per plant, MS1P-Weight of seeds per plant, M1000S-Weight of 1000 seeds, NS1P-Number of seeds per pita , VEGP.- Vegetation period/ number of days, PBUT-Period from emergence to budding/number of days, PNC-Period from emergence to phase beginning of flowering/number of days, PCAF-Period from emergence to mass flowering phase/number of days .
The Krasi CLP hybrid is characterized (table 3, figure 1.) by a plant height of 164 cm, a seed diameter of 21 cm. The leaf area of the hybrid has an area of 2445.6 cm2 and 28 asymmetrically arranged leaves. The mass of 1000 seeds is 60.5 g, with a plant mass of 114.6 g and 2245 seeds per pita. The hybrid is medium-early with a vegetation period of 110 days, the period from germination to budding is 38 days and 70 days is the period from germination to mass flowering. The flowering period from the beginning to the end of flowering is 13 days. The production potential depends on the conditions of the year and varies from 328kg to 350kg. The hybrid is characterized by good drought tolerance, resistance to downy mildew 731, phoma, phomopsis, alternaria and Orobanche cumana.

Figure 1. Hybrid Krasi CLP

Biochemical and phytopathological characteristics of Krasi CLP hybrid

Table 4. Biochemical and phytopathological characteristics of Krasi CLP hybrid

Hibyd/ Krasi CLP	***Plasmopara helianthi* /731/ attack %**	***Phoma macdonaldii* %**	***Phomopsis helianthi* attack %**	**Sclerotinia sclerotiorum attack root, stem%**	**Sclerotinia sclerotiorum attack head %**	***Orobanche cummana* attack % %**
	8,8	31,2	4	18,4	0	18,9

From the phytopathological studies (table 4.) of the hybrid Krasi CLP in IASAS, the hybrid is characterized by very good resistance to downy mildew /731/ 8.8%, Phomopsis - 4%, sclerotinia attack pita - 0%, and average resistance to sclerotinia root and stem 18, 4%, phoma-31.2% and to the blue wrist parasite-18.9%.

CONCLUSIONS

Hybrid Krasi CLP was created at the Dobrudja Agricultural Institute - General Toshevo and was approved for recognition and registration in the Republic of Bulgaria under the RHS by order RD-12-3/17.03.2023. in the official variety list of the country by order. of the Minister of MFA of the Republic of Bulgaria. Hybrid Krasi CLP is a new Bulgarian two-line sunflower hybrid suitable for cultivation according to Clearfield Plus technology, obtained by inter-line hybridization. The hybridization involved the maternal sterile line 1111A as a component and the fertility restorer line GTS 51R. The two parental lines have very good general and specific combinatorial ability. Hybrid Krasi is medium-early with a vegetation period of 109-111 days, seed diameter 19-21cm, mass of 1000 seeds 62g, kernel content %-77%. The production potential, depending on the conditions of the year, varies from 328 kg to 350 kg. The oil is of the linoleic type and its content varies from 49-51%. The hybrid features good drought resistance, resistance to downy mildew 731, phoma, phomopsis, Alternaria and Orobance Cumana.

REFERENCES

Chander S and Sheoran R K 2014. Studies on correlation and path coefficient analysis for seed yield in sunflower hybrids (Helianthus annuus L.). Proceedings of National Seminar on Reorientation of Agricultural Research to ensure National Food Security, Jan 6-7, 2014, Directorate of Research, CCS HAU, Hisar, pp. 63.

Chander S, Sheoran RK. Studied on correlation and path coefficient analysis for seed yield in sunflower hybrids (Helianthus annuus L.)2014. Proceedings of National Seminar on Reorientation of Agricultural Research to ensure National Food security Directorate of Research, CCS HAU, Hisar. 2014, 63.

Chandirakala R, Ameena P, Manivannan N. Metroglyphanalysis on genetic diversity in germplasm accessions ofsunflower (Helianthus annuus L.). Journal of OilseedsResearch. 2015; 32(2):170-173

Georgiev, 2016 . PRODUCTION POTENTIAL OF NEW SUNFLOWER HYBRIDS DEVELOPED AT DOBRUDZHA AGRICULTURAL INSTITUTE GENERAL TOSHEVO. 19th International Sunflower Conference, Edirne, Turkey, 2016.

Hladni, N., Skoric, D., Kraljevic-Balalic, K., Sakac, Z., and Jovanovic, D. (2006). Combining ability for oil content and its correlation with other yield components in sunflower (H. annuus L.). Helia, 29(44):101-110.

Kaya, Y., Evci, G., Durak, S., Pekcan, V., and Gucer, T. (2007). Determining the relationships between yield and yield attributes in sunflower. Turk. J.Agric For., 31:237-244

Kumari and Sheoran (2012). Genetic variability, heritability and character association in sunflower. Research on Crops 13(2):566-57

Mijic, A.,I. Liovic, Z. Zdunic, S. Maric, A. Marajanovic-jeromela, M. Jankulovska (2009): Quantitaive analisys of oil yield and its components in sunflower (*Helianthus annuus* L.). Roman. Agric. Res., 41-46.

Prabhakaran, V.; Reddy, S. M.; Reddy, T. D.; Ranganatha, A. R. G. (2013). Character association and path analysis in sun-flower (Helianthus annuus L.). Journal of Oilseeds Research, 30(1), 65-67

Satjawattana, K. (2005). Development of single crosses of sunflower for high yield and oil percentage, [Ph.D. thesis]. School of Crop Production Technology, Institute of Agriculture, Suranaree University of Technology, Nakhon Ratchasima, Thailand, p. 106.

Singh 2018. Correlation analysis for seed yield and its component traits in sunflower. Journal of Pharmacognosy and Phytochemistry 2018; 7(3): 2299-2301

Skoric, D., 1975. Possibilities of using heterosis based on male sterility of sunflower. Ph.D. thesis. University of Novi Sad, Agriculture Faculty, pp.1-148. (In Serian)

Srimuenwai, P. (2006). Improvement of agronomic characters, oil content and yield of synthetic varieties of sunflower, [M.Sc. thesis]. School of Crop Production Technology, Institute of Agriculture, Suranaree University of Technology, Nakhon Ratchasima, Thailand, p. 80.

Sujatha & Nadaf 2013. Correlation for Yield and Yield Related Trait in Mutant and Segregating Genotypes in Sunflower (Helianthus annus L.). Molecular Plant Breeding, 2013, Vol. 4, No. 32 doi: 10.5376/mpb.2013.04.0032.

Williams,J. (2008). Greenhouses for the growth region. Journal of Environmental Planning & Management 51(1), 1-34. ISSN: 1360-0559

ECOSYSTEM SERVICES OF ORGANIC AGRICULTURE TO THE ENVIRONMENT AND SOCIETY (CASE STUDY: TÜRKİYE)

Mehmet Uğur YILDIRIM [1], Hossein KAZEMI [2], Ercüment Osman SARIHAN[1], Tuba ARJUMEND[3]*

[1]*Uşak University, Faculty of Agriculture, Department of Field Crops, Uşak, Türkiye*

[2]*Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*

[3]*Usak University, Faculty of Agriculture, Department of Plant Protection, Türkiye*

Corresponding author e-mail: ugur.yildirim@usak.edu.tr

ABSTRACT

The current trend of decline in the ability of agroecosystems (e.g. organic farming) to provide ecosystem services is a major threat to food security worldwide. In 2022, the total area of organic agriculture in Türkiye totaled about 310.584 ha from 24 million ha of agriculture area. On the other hand, Türkiye is facing environmental problems such as soil degradation, deforestation, climate change, loss of biodiversity, etc, which pose major threats to human safety, health, environment, and productivity. Therefore, organic farming can provide important services to the environment and society. Basically, the function of organic farming systems plays an important role in both ecological and economic aspects by improving livelihoods, protecting biodiversity and increasing soil fertility and health. The development of organic farming is possible for the use of its services such as provisioning, regulating, cultural, and supporting services. For example, organic farming provides a systems approach to reducing greenhouse gases (GHG) emissions and increasing soil carbon sequestration while maintaining healthy soils and protecting biodiversity. This can reduce the world's agricultural GHG emissions by about 20%. Furthermore, this agricultural system may cut soil erosion by 22%, which is an issue that Turkiye's agricultural lands are facing. In general, organic farming reduces negative impacts on nature-related ecosystems while simultaneously providing ecosystem services. In this article, we will address this issue, how can the development of organic agriculture minimize the environmental problems of Türkiye by providing ecosystem services.

Keywords: Agriculture, biodiversity, ecosystem services, organic farming, Türkiye.

INTRODUCTION

By 2050, the world's food production will need to rise by 60% due to an expanding population, present food distribution patterns, and food consumption habits (United Nations Environment Programme, 2012). Agricultural practices can assist in achieving the goals by reducing trade-offs between output and the environment and by identifying and increasing the ecosystem services (ES) from farmlands (United Nations Environment Programme, 2014). Ecosystem services (ES) like organic farming are essential to the ongoing provision of food to an expanding global population, yet food and nutritional security are under risk due to the rising use of chemical fertilizers globally. Organic agriculture is essentially a method of farming that stays away from artificial fertilizers, pesticides, and genetically modified organisms. Rather, it depends on organic techniques like crop rotation and composting to preserve soil fertility and biological pest management (Daylam et al., 2023). The performance of organic farming systems plays an important role in both ecological and economic aspects by improving livelihoods, protecting biodiversity, increasing soil fertility and reducing poverty. In 2022, the total area of organic agriculture in Türkiye estimated about 310.584 ha from 24 million ha of agriculture area (Anonymous, 2024). Turkiye is dealing with environmental issues including deforestation, soil degradation, climate change, biodiversity loss, etc. These issues pose serious risks to people's health, safety, and productivity and impede the pursuit of sustainable development. Thus, organic farming in Turkiye may offer significant benefits to both the environment and society.

The aim of the paper was the assessment of ecosystem services of organic agriculture to the environment and society of Türkiye. In this paper, we answer this question, how can the development of organic agriculture minimize the environmental problems of Türkiye by providing ecosystem services.

MATERIALS AND METHODS

We first investigated the state of agriculture in Türkiye based on official reports, statistics, and available sources. Then, we recorded the problems in the agricultural sector of this country. Next, we looked at the scientific literature on organic farming and determined the environmental services it provides. In the end, to solve the existing problems in Türkiye, we suggested some solutions based on organic agriculture services and linked them together.

To address our research objective, we reviewed the literature and analyzed the assessment of ecosystem services related to organic farming. Scientific databases like Science Direct and Scopus were used to gather publications, which were then investigated and categorized. All data was calculated and classified into 4 groups, including provisioning, regulating, supporting, and culture services.

RESULTS AND DISCUSSION

The growth of organic agriculture in Türkiye is possible through the use of its services such as provisioning, regulating, cultural, and supporting services Table 1). The country is facing major environmental problems such as soil degradation, deforestation, climate change, loss of biodiversity, etc, which pose major threats to human safety, health, environment, and productivity. Therefore, organic farming in this country can provide important services to the environment and society. For example, organic agriculture can improve the quality and health of Türkiye's agricultural products, which in turn improves the health of the society. Agriculture continues to be a significant economic sector in Türkiye. Wheat, sugar beet, milk, poultry, cotton, hazelnuts, apricots, oregano, vegetables, and fruits are the main goods (IFAD, 2023: ITA, 2022). Sustainable food and fiber production in agriculture depends on ecosystem services like pollination, soil formation, biological control, and nutrient cycling. In order to provide ecosystem services, organic agriculture needs a comprehensive approach that acknowledges the interdependencies between ecological processes, agricultural output, and human welfare. Therefore, maintaining and promoting organic agriculture is important to achieve sustainable production and economic conditions (Padmavathy and Poyyamoli, 2011).

Table 1. Services by organic farming to agriculture of Türkiye

Services by organic farming	Service type	Problems related to agriculture
High quality food	Provisioning	Food production with low quality
Healthy food	Provisioning	Unhealthy food production
Help to food security	Provisioning	Food with pesticide remains
Help to pollination	Regulating	Decrease of pollinators
Soil protect	Regulating	Soil erosion
Biodiversity protect	Supporting	Loss biodiversity
Soil fertility	Regulating	Low organic matter
Soil healthy	Regulating	Land degradation
Protect of water bodies	Regulating	Water pollution
Carbon sequestration	Regulating	Greenhouse gases emission
Protect of environment	Regulating	Environmental pollution
Pest control without chemicals	Regulating	High consumption of pesticides
Increase of soil organic matter	Regulating	High consumption of chemical fertilizer
Agrotourism	Culture	Low income

Turkiye reported 558 Mt of GHG emissions for 2022, which is higher than what a global carbon budget would permit. Additionally, the country's gross domestic product in 2022 was 6.6 t, greater than the global average (Turkstat, 2024; Ritchie and Roser, 2020). Additionally, research conducted in Turkiye calculated that almost one-third of all national emissions are related to food production (Üçtuğet al., 2021). The agriculture industry in Turkey has a part to play in either escalating or resolving this worldwide challenge. Organic farming, on the other hand, provides a systems approach to lowering greenhouse gas (GHG) emissions and raising soil carbon sequestration while preserving biodiversity and healthy soils. This has the potential to cut global agriculture GHG emissions by almost 20% (IFOAM, 2022).

Soil erosion is a challenge faced by Turkish farmers (Table 1), with estimates of moderate to severe erosion being 59% in agricultural regions, 54% in forest lands, and 64% in rangelands.

Because of its geographical position and different climate characteristics, Türkiye is one of the countries most affected by drought and desertification (General Directorate of Combating Desertification and Erosion, 2021). According to the findings of research, Turkiye has lost almost 287.5 million tons of soil in a year (Berberoglu, et al., 2020). There are many reports that organic farming helps to control soil erosion by conserving water and soil resources. Organic agricultural systems can reduce soil erosion by 22% and protect water resources by lowering nitrate leaching. Generally, enhancing soil structure in organic farming makes it more resistant to climate change, less prone to erosion, and supportive of plant health (IFAOM, 2022). Therefore, this agricultural system offers beneficial services while minimizing detrimental effects on ecosystems connected to nature including soil protection and biodiversity.

In the year 2023, Türkiye's agriculture sector utilized 57.8 thousand tons of pesticides. Over 19,600 tons of fungicides were utilized this year, making them the most popular kind of pesticide. Herbicides come next, with over 15,000 tons of usage in 2023 (Statista, 2024). The main environmental concerns related to pesticides are contamination of soil, water, and air damage to non-target organisms, and the health of humans. Also, these conventional agriculture systems have negative impacts on neighboring natural ecosystems. According to reports, the ban on synthetic fertilizers and pesticides along with biodiversity-boosting practices like varied crop rotations including legumes, landscape features, and less tillage, leads to an average of 30% greater species and 50% more individuals residing in organically managed areas (IFAOM, 2022). In organic farming, biological control of pests and diseases, as opposed to chemical pesticides, is the most sustainable and environmentally friendly technique to manage agricultural fields. Thus, organic farming provides advantages like biodiversity, environmental protection, and human health while simultaneously lessening harm to ecosystems that are a part of the natural world (Kuhling and Trautz, 2013).

With a nearly 34% endemism rate, Türkiye boasts the most diverse flora of any temperate region. This richness is increasing even more with the newly identified plant species. Approximately one thousand endemic plant species are included in the endangered species list. Also, 305 key biodiversity areas are identified in Türkiye (Türkiye’s Biodiversity, 2024). The current agriculture practices have led to a decrease in biodiversity in Türkiye (Table 1). Loss of biodiversity may have hidden consequences that make ecosystems more vulnerable to a variety of biotic and abiotic stressors (Winqvist et al., 2012). Organic farming has led to a rise in the quantity and species richness of many common species; nevertheless, the effects are often species-specific and tissue- or trait-dependent. Furthermore, maintaining a sustainable agricultural system and promoting biodiversity and high-quality soil through land management methods are key components of organic agriculture's sustainable food production strategy. Feledyn-Szewczyk et al. (2016) criticized the stepping up production and increased use of chemical pesticides, fertilizers, energy, and water in agricultural ecosystems. They reported that agricultural lands globally need further biological and agronomic methods to produce food and fiber. Principally, biodiversity conservation is important for the suitable function of ecosystems and the provision of services. A high level of biodiversity in agricultural ecosystems contributes to the sustainability and economic viability of agricultural output.

In this paper, we addressed the subject of how ecosystem services provided by organic agriculture might help to mitigate Turkiye's environmental issues. The performance of organic farming in this country can play important roles in the ecological, environmental, and economic aspects by improving livelihoods, protecting biodiversity, increasing soil fertility and health, and reducing pollution and poverty.

REFERENCES

Berberoglu, S., Cilek, A., Kirkby M., Irvine, B. and Donmez, C. 2020. Spatial and temporal evaluation of soil erosion in Türkiye under climate change scenarios using the Pan-European Soil Erosion Risk Assessment (PESERA) model. Environment Monitoring and Assessment. 192:1-22.

Anonymous 2024. https://cevreselgostergeler.csb.gov.tr/organik-tarim-alanlari-ve-uretim miktarlari-i-85837 (accessed 30.08.2024)

Daylam, F., Kazemi, H. and Kamkar, B. 2023. Modelling organic farming suitability by spatial indicators of GIS integrated MCDA in Golestan Province, Iran. NJAS Impact in Agricultural and Life Sciences. 95(1) 10.1080/27685241.2023.2191796.

Feledyn-Szewczyk, B., Kuś, J., Stalenga, J., Berbeć, A.K. and Radzikowski, P. 2016. The role of biological diversity in agroecosystems and organic farming. In: Konvalina, P.: Organic Farming - A Promising Way of Food Production. Intech Publisher.

General Directorate of Combating Desertification and Erosion, 2021. Desertification and Erosion in Turkey. https://webdosya.csb.gov.tr/db/cem/icerikler/cemxbrosxingxmail-20211108120330.pdf.

IFAD, 2023. Türkiye. International Fund for Agricultural Development. https://en.wikipedia.org/wiki/International_Fund_for_Agricultural_Development.

IFOAM (International Federation of Organic Agriculture Movements). 2024. What is organic agriculture? IFOAM.

ITA, 2022. Turkey – Country Commercial Guide". International Trade Administration, U.S. Department of Commerce.

Kuhling, I. and Trautz, D. 2013. The role of organic farming in providing ecosystem services. International Journal of Environmental and Rural Development. 4(1): 175-178.

Padmavathy, A. and Poyyamoli, G. 2011. Effects of conventional and organic management strategies on soil quality and biodiversity in agricultural fields of Bahour, Puducherry, India. American Eurasian Journal of Sustainable Agriculture. 10 (4):644-652.

Ritchie, H., and Roser, M. 2020. CO2 and Greenhouse Gas Emissions. Our World in Data. https://ourworldindata.org/co2/country/turkey.

Statista. 2024. https://www.statista.com/statistics/1457138/turkey-pesticide-used-in-agriculture-by-type/#:~:text=In%202023%2C%20a%20total%20of,15%2C000%20tons%20used%20in%202023.

Turkstat.2024. Greenhouse Gas Emissions Statistics, 1990-2022". https://data.tuik.gov.tr/Bulten/Index?p=Greenhouse-Gas-Emissions-Statistics-1990-2022-53701&dil=2.

Turkey's Biodiversity. 2024. https://dogadernegi.org/en/turkeys-biodiversity/

United Nations Environment Programme 2012. Avoiding future famines: strengthening the ecological foundation of food security through sustainable food systems. Nairobi: UNEP. A UNEP Synthesis report

United Nations Environment Programme 2014. Food security and biodiversity: challenges, conflicts and options, UNEP Policy Series No. 14. Nairobi: UNEP.

Üçtuğ, F.G.; Günaydin, D., Hünkar, B. and Öngelen, C. 2021. Carbon footprints of omnivorous, vegetarian, and vegan diets based on traditional Turkish cuisine. Sustainable Production and Consumption. 26: 597–609.

Winqvist, C., Ahnström, J. and Bengtsson, J. 2012. Effects of organic farming on biodiversity and ecosystem services: taking landscape complexity into account. Annals of the New York Academy of Sciences. https://doi.org/10.1111/j.1749-6632.2011.06413.

EFFECT OF IRRIGATION REGIME, NITRATE NITROGEN FERTILIZATION, APPLICATION PHASE AND GENOTYPE ON QUALITATIVE AND QUANTITATIVE PARAMETERS IN COMMON WHEAT FOR INCLUSION IN AN ALGORITHM OF THE SYSTEM FOR INTELLIGENT MANAGEMENT OF AGRICULTURAL PROCESSES

Evgeniy DIMITROV, Stanislav STAMATOV, Teodora ANGELOVA, Zlatina UHR
IPGR "K. Malkov", Sadovo, Agricultural Academy, Bulgaria

ABSTRACT

The drip irrigation has been used in recent years as a water and fertilizer saving technology. A three-year field experiment was conducted in the selection field of IPGR, Sadovo, in the period 2021-2023. The response of the two varieties of wheat to different irrigation rates and levels of nitrate nitrogen fertilization was tested. Eighteen fertilizing treatments were used in the experiment, including one-time, two-time and three-time application of the nitrogen fertilizer rate (17 kg N) in combination with a different irrigation regime - 10, 20 and 30 mm. Fertilizer and irrigation rates were applied in the stages of tillering, end of tillering, beginning of stem elongation and stem elongation. Grain virtuousness response and wet gluten yield were monitored. The obtained results show that the highest values of the virtuousness trait were obtained in the varieties Geya 1 and Sachets with an irrigation rate of 20 mm in combination with single and double application of nitrogen fertilization. In the wet gluten yield and its quality characteristics, the best results are available with the Sachets variety in the version with three times the application of the fertilizer and the maximum irrigation rate. The greatest influence on the traits of vitreousness and gluten relaxation is exerted by the interaction of genotype x the tested factors, with the rest - yield of wet gluten, dry gluten yield - the influence of the genotype is the leading factor. The year of cultivation is decisive for the number of baker's strength. Quality traits in wheat are controlled by a smaller number of genes, and the influence of nitrogen fertilization and irrigation rates in the set periods is weaker on their expression. The mathematical processing of the data was carried out using the program Microsoft Excel for Windows. The obtained results will be integrated into an algorithm of the system for intelligent management of agricultural processes.

Key words: Smart farming, Common winter wheat, quality, Vitreous, Wet gluten yield

INTRODUCTION

The wheat (*Triticum aestivum* L.) is one of the main cereal crops cultivated worldwide (Allami, et al., 2020; Bielski, et al., 2021; Erenstein, et al., 2022; Reinhardt, et al., 2022). It is a major source of plant proteins that depend on the unique characteristics of grain storage proteins (Shewry et al., 2009). Provides the human diet with essential amino acids, minerals, vitamins, as well as beneficial dietary fiber (Khalid et al., 2023)

Due to its high plasticity and easy adaptation to different environmental conditions, wheat has a significant advantage in terms of global food security over other cereals, as it can be grown under different climatic conditions. However, biotic and abiotic factors strongly influence wheat grain yield and quality (Allami et al., 2020; Pawlak et al., 2020; Behnassi et al., 2022; Mottaleb et al., 2022; https://ec.europa.eu/eurostat/statistics-explained/). Dry seasons, high temperatures, water deficit are the main abiotic factors that reduce yield and affect the quality of wheat grain. In the context of global water scarcity and climate change, the sustainability of wheat cropping systems is a major challenge for scientists and farmers (Ding et al., 2018; De Oliveira Silva et al., 2020b; Zubair et al., 2021; Zingale et al., 2022).

In dry land areas, which cover approximately 41% of the earth's surface, water stress has a significant negative impact on the crop (Zain et al., 2023; Amirahmadi et al., 2024). It is the second most irrigated cereal crop. after the rice (Frenken and Gillet, 2012). Nevertheless, the large amounts of water used in conventional irrigation methods lead to an irrational use of limited water resources.

Their shortage and unwise application of nitrogen fertilizers is a problem in wheat production (Yao et al., 2023). The application and appropriate management of nitrogen (N) fertilizer is also critical for wheat plant growth and yield formation and improvement of quality (Peng et al., 2006; Dalin et al., 2017; Shahdany et al., 2018). Soil water and nitrogen resources suffer from overuse. Management of these resources is therefore necessary for sustainable agricultural productivity (Yao et al., 2023).

Studies on the physiological and biochemical processes of common winter wheat under different levels of irrigation and nitrogen fertilization are needed to better understand winter wheat growth and yield pattern and quality change under different soil and environmental conditions (Zain et al., 2023).

Therefore, various irrigation systems have been applied to further improve wheat yield and quality characteristics and water use efficiency, such as one-time pre-sowing irrigation (Sun et al., 2019), supplementary irrigation based on moisture content measurement (Meng et al., 2015), integration of micro-sprinkler irrigation (Li et al., 2019b), use of drip irrigation systems (Hoff et al., 2018; Qu et al., 2022; Bouazzama et al., 2024).

Irrigation systems used and nitrogen (N) fertilization regimes have complex effects on wheat physiology, growth and development, leading to regulation of wheat grain yield and quality (Hamani, et al., 2024).

The use of drip irrigation is recommended to deal with water shortage problems. It appears to be highly effective in regulating soil moisture at a capacity of 50-60% (Wang et al., 2013; Zhang et al., 2013), and compared to surface irrigation methods, it can improve water use efficiency (Mehmood et al., 2019). Wang et al. (2013) reported a 5–13% improvement in wheat grain yield obtained with drip irrigation compared to gravity.

In the current climate challenges, an integrated approach of applying water and nitrogen through a drip irrigation system is crucial (Hamani, et al., 2024). Common winter wheat grown for consumption should be characterized by yield and high technological quality (Guerrini et al., 2020; Dilmurodovich et al., 2022). The suitability of the grain for processing is determined by the quality characteristics of the grain; the physical properties of the grain that affect its milling (vitreousness, hardness, bulk density, weight of 1000 grains and the protein complex (Rasheed et al., 2014; Lachutta et al., 2024) The protein content of the grain has a significant influence on the quality of the flour (Li et al., 2018; Kumar et al., 2021).

Storage proteins (gliadins and glutenins) play an important role during dough formation and are responsible for the baking qualities of wheat flour (Zhang et al., 2007; Li et al., 2008; Li et al., 2021; Hao et al., 2023). These two proteins during the mechanical kneading of flour in the presence of water combine to form a viscous and elastic mass and a plastic mass -gluten. Gluten determines the water absorption capacity of the flour and therefore the elasticity and plasticity of the resulting dough (Wieser et al., 2007; Dizlek et al., 2022; Zhang, et al., 2022)

MATERIAL AND METHOD

Plant material

Two of the institute's varieties participated in the test - Geya 1 - medium-strength wheat (Group B) and Sashets - strong wheat (Group A).

Setting up the experiment

The experiment was carried out on an experimental field of IPGR, Sadovo in the 2021 - 2023 harvest years. The response of two varieties of common winter wheat to three applications of nitrogen fertilization and different application rates was tested.

The reaction of grain vitreous,% (BDS 13378:1976), wet gluten yield, % (BDS EN ISO 21415-1:2007), gluten release, mm (BDS 13375:1990/Amendment 1:1993), was monitored. number number of bakery strength, acc. unit (BDS 13375:1990/Amendment 1:1993) and dry glugen (%) BDS EN ISO 21415-4:2007.

The nitrogen fertilizer rate (17 kg nitrate nitrogen) was applied once, twice and three times in combination with three different irrigation rates – 10, 20 and 30 mm. Fertilizer and irrigation rates were applied in the stages of tillering, end of tillering, beginning of stem elongation and elongation.

The two varieties of the institute were fertilized with ammonium nitrate at a dose of 17 kg/da and distributed as follows:

1. The entire amount introduced in the tillering-beginning of stem elongation phase.
2. ½ of the amount entered in the tillering-beginning of stem elongation phase, and ½ entered in the stem elongation phase.
3. 1/3 of the amount entered in the tillering-beginning of stem elongation phase, 1/3 entered in the tillering phase and 1/3 entered in the heading phase.

The amount of water supplied to the experimental variants was 10, 20 and 30 l/m2 in the stages of twining - beginning of spinning and leveling. The experience set in this way formed 18 test plots with a total harvest area of 1 da.

By means of the coefficient of variation, the variation of some indicators was determined (Dimova and Marinkov, 1999) as: weak - up to 10%, medium - greater than 10% and less than 20%, strong - over 20%.

RESULTS AND DISCUSSION

The Table 1 presents the obtained results for the vitreousness of the grain. The vitreousness of the grain is one of the main quality indicators. It is related to its density, reflects the structural-mechanical properties of endosperm and the ratio of protein to starch. Differences in endosperm structures were found in vitreous and mealy grains. Vitreousness is a physical indicator that is extremely influenced by the climatic conditions during the year of cultivation, the genotype, as well as the method of cultivation and their interaction. (Filipov, 2004; Cacak-Pietrzak, 2008; 2011; Brankovic et al., 2014; Mitura et al., 2023).
On average for the study period, the best values of the indicator were obtained with the Geya 1 variety, variants with three times the fertilizer rate and irrigation, both with 10 and 20 mm of water. In the case of variety Sachets /group A/, they are the highest when nitrogen is applied twice and 20 mm of water, as well as when feeding three times and thirty mm of water. Vitrification is an indicator that is most influenced by environmental conditions (Angelova et al., 2023). A decrease in the values of the indicator is observed when the irrigation rate is increased. The lowest values were recorded for the Sachets variety with a single application of the fertilizer and the highest irrigation rate of 30 mm. The coefficient of variation of the indicator is average according to the scale of Dimova and Marinkov, - 10.9%.

Table 1. Vitreousness of grain for the period 2021-2023

№	Variety	2021	2022	2023	X	N, g	N, g	N, g	Irrigation rate, mm
1	Geya	50	48	56	51.	500	-	-	10
2	Sachet	32	50	40	40.	500	-	-	10
3	Geya	44	42	48	44.	500	-	-	20
4	Sachet	41	42	48	43.	500	-	-	20
5	Geya	42	48	34	41.	500	-	-	30
6	Sachet	28	36	38	34.	500	-	-	30
7	Geya	54	44	44	47.	250	25	-	10
8	Sachet	44	43	40	42.	250	25	-	10
9	Geya	28	28	50	35.	250	25	-	20
1	Sachet	54	56	44	51.	250	25	-	20
1	Geya	48	43	40	43.	250	25	-	30
1	Sachet	40	47	44	43.	250	25	-	30
1	Geya	54	50	40	48.	166	16	16	10
1	Sachet	54	43	38	45.	166	16	16	10
1	Geya	52	55	46	51.	166	16	16	20
1	Sachet	44	53	40	45.	166	16	16	20
1	Geya	38	49	44	43.	166	16	16	30
1	Sachet	38	56	50	48.	166	16	16	30
Mean		43.	46.	43.	44.				
Minimum		28	28	34	34.				
Maximu		54	56	56	51.				
Stand. deviation					4.8				
Coeff. of variation					10.				
Stand. error of mean					1.1				

On average for the period, we report the highest values of wet gluten yield and dry gluten (Table 2) for the Sachets variety with the triple nitrogen application and irrigation regime with 20 and 30 mm of water - option 16 and 18., and for Gaia 1 with option 11 and option 17. The lowest results are for Sachets variant with single fertilization and maximum irrigation rate. The variation in the yield of wet gluten is low and that of dry gluten is medium. This study showed that the water use efficiency of medium-gluten wheat increased with the increase in fertilization frequency, while that of high-gluten wheat was the opposite and was in confirmation of that obtained by Hao et al., 2023. In mature wheat grains, gliadins and glutenins are the main water-insoluble proteins forming the gluten complex (gluten). They represent 80 - 85% of wheat proteins (Van Der Broght, 2005; Zlateva, 2010). They are responsible for the elasticity and extensibility of the dough, which in turn determines the processing qualities of various end products.(Rasheed et al., 2014). Wet gluten in wheat grain depends on a number of factors, such as genotype, environmental conditions during pouring and ripening of the grain. (Karadzhov et al., 1998), Ivanova and Kirchev, 2018; Biel et al., 2020; Campesi et al., 2023; Altenbach et al., 2012; Zhen et al., 2020). Irrigation has a significant impact on the yield and quality of wheat. Supplemental irrigation at critical growth stages of wheat increased crop yield to face extreme drought events (Campesi et al., 2023; Zhang et al., 2019). Flour protein content increases significantly under water deficit, especially total gluten content, due to higher nitrogen accumulation and lower carbohydrate accumulation (Liu, et al., 2022).

Table 2. Wet gluten yield and dry gluten values for the period 2021-2023

№	Variety	Value								Nutritional and watering regime			
		Wet gluten yield				Dry gluten							
		2021	2022	2023	X	2021	2022	2023	X	N, g	N, g	N, g	Irrigation rate, mm
1	Geya 1	32.4	25.9	32.8	30.4	9.9	8.6	9.4	9.3	500	-	-	10
2	Sachets	30.5	27.8	28.4	28.9	9.2	8.6	8.4	8.7	500	-	-	10
3	Geya 1	27.8	26.4	28.0	27.4	8.5	8.4	7.7	8.2	500	-	-	20
4	Sachets	31.2	25.8	30.3	29.1	9.6	8.5	8.7	8.9	500	-	-	20
5	Geya 1	31.6	26.7	26.0	28.1	9.7	8.0	7.8	8.5	500	-	-	30
6	Sachets	27.8	27.0	22.4	25.7	8.4	8.4	6.7	7.8	500	-	-	30
7	Geya 1	33.6	27.7	29.2	30.2	10.7	8.7	8.2	9.2	250	250	-	10
8	Sachets	28.9	26.8	27.2	27.6	9.4	8.8	7.6	8.6	250	250	-	10
9	Geya 1	26.2	28.0	24.8	26.3	8.2	8.1	7.8	8.0	250	250	-	20
10	Sachets	38.8	30.5	28.8	32.7	12.1	10.1	8.5	10.3	250	250	-	20
11	Geya 1	39.0	32.5	26.8	32.8	12.4	11.2	8.7	10.8	250	250	-	30
12	Sachets	34.9	35.9	29.6	33.5	11.2	11.8	9.7	10.9	250	250	-	30
13	Geya 1	36.6	30.7	26.6	31.3	11.5	10.0	7.9	9.8	166	166	166	10
14	Sachets	38.3	32.3	27.5	32.7	12.2	10.6	8.5	10.4	166	166	166	10
15	Geya 1	35.2	35.1	30.6	33.6	11.4	11.6	8.7	10.6	166	166	166	20
16	Sachets	38.2	31.9	28.6	32.9	11.9	11.8	9.4	11.0	166	166	166	20
17	Geya 1	37.5	31.6	30.5	33.2	11.8	10.5	9.8	10.7	166	166	166	30
18	Sachets	36.3	34.2	33.2	34.6	11.3	11.5	10.6	11.1	166	166	166	30
Mean		**33.6**	**29.8**	**28.4**	**30.6**	**10.5**	**9.7**	**8.6**	**9.6**				
Minimum		**26.2**	**25.8**	**22.4**	**25.7**	**8.2**	**8.0**	**6.7**	**7.8**				
Maximum		**39.0**	**35.9**	**33.2**	**34.6**	**12.4**	**11.8**	**10.6**	**11.1**				
Stand. deviation					**2.8**	**Stand. deviation**			**1.1**				
Coeff. of variation					**9.1**	**Coeff. of variation**			**11.9**				
Stand. error of mean					**0.7**	**Stand. error of mean**			**0.3**				

The physical properties of the dough largely depend on the quantity and quality of gluten (Hristov, 1967). The quality of the wet gluten of wheat is determined by the relaxation of the wet gluten. It represents the difference (in mm) between the initial and final diameter of the gluten ball with a mass of 4g after a 60-minute stay at a temperature of 30°C (Zlateva, 2010). A good allowance for breadmaking is 6 to 10 mm. In the present study, the release of gluten in both varieties, as well as in the three years of the study, was above the norms, i.e. higher than the maximum value of 10 mm. The release of the gluten of the Geya 1 and Sashets varieties for the period is from 12.7 - 19.5 mm (Table 3). The highest values were recorded with a single fertilization and watering of 20 mm in both varieties. The trait number of bakery strength ensures the level of bakery quality - volumetric yield of bread with good dimensional stability (Belcheva, 2018). The number of bakery strength as a complex indicator expressing the quantity and quality of gluten, although weaker, also changes depending on mineral fertilization (Nenova and Stoyanova, 2011).

Table 3. Gluten release and number of bakery strength values for the period 2021-2023

№	Variety	Value								Nutritional and watering regime			
		Gluten release				Bakery power number							
		2021	2022	2023	X	2021	2022	2023	X	N, g	N, g	N, g	Irrigation rate, mm
1	Geya 1	22.5	8.0	28.0	19.5	40	56	40	45,3	500	-	-	10
2	Sachets	15.0	14.0	27.0	18.7	46	46	40	44,0	500	-	-	10
3	Geya 1	21.0	13.0	23.0	19.0	40	46	39	41,7	500	-	-	20
4	Sachets	25.5	8.0	25.0	19.5	40	56	40	45,3	500	-	-	20
5	Geya 1	15.5	13.0	24.0	17.5	46	47	40	44,3	500	-	-	30
6	Sachets	20.5	10.5	26.5	19.2	41	51	35	42,3	500	-	-	30
7	Geya 1	19.0	11.0	18.0	16.0	42	51	42	45,0	250	250	-	10
8	Sachets	15.5	12.0	19.0	15.5	45	48	41	44,7	250	250	-	10
9	Geya 1	14.5	20.5	10.0	15.0	45	41	49	45,0	250	250	-	20
10	Sachets	18.0	10.0	21.0	16.3	43	55	40	46,0	250	250	-	20
11	Geya 1	18.0	12.0	15.0	15.0	43	52	44	46,3	250	250	-	30
12	Sachets	17.5	11.0	14.0	14.2	44	55	47	48,7	250	250	-	30
13	Geya 1	14.0	17.0	24.0	18.3	48	44	40	44,0	166	166	166	10
14	Sachets	15.5	11.0	21.0	15.8	47	54	40	47,0	166	166	166	10
15	Geya 1	11.0	11.5	23.0	15.2	55	54	40	49,7	166	166	166	20
16	Sachets	15.0	8.0	15.2	12.7	47	65	45	52,3	166	166	166	20
17	Geya 1	17.5	14.0	9.5	13.7	44	48	57	49,7	166	166	166	30
18	Sachets	15.5	9.0	15.0	13.2	47	62	46	51,7	166	166	166	30
Mean		**17.3**	**11.9**	**19.9**	**16.4**	**44.6**	**51.7**	**42.5**	**46.3**				
Minimum		**11.0**	**8.0**	**9.5**	**12.7**	**40**	**41**	**35**	**41.7**				
Maximum		**25.5**	**20.5**	**28.0**	**19.5**	**55**	**65**	**57**	**52.3**				
Stand. deviation					**2.3**	**Stand. deviation**			**3.0**				
Coeff. of variation					**13.8**	**Coeff. of variation**			**6.5**				
Stand. error of mean					**0.5**	**Stand. error of mean**			**0.7**				

Vitreousness is an traait that is not significantly affected by the amount of irrigation water (Figure 1). This is evident from the resulting linear equation of the empirical data, it was obtained with a low coefficient of determination. The exponential equation shows that at a water supply between 20 and 25 mm, the vitreousness values are the highest for both varieties.

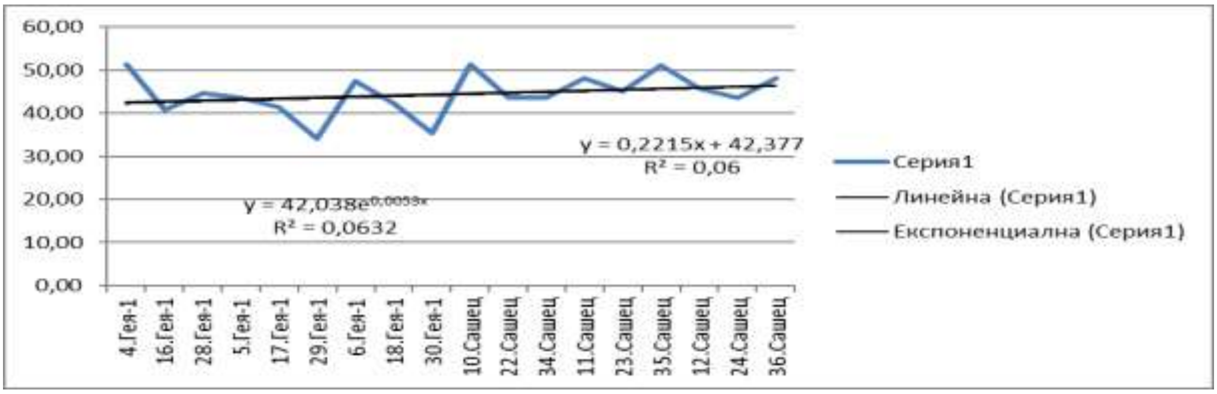


Figure 1. Effect of irrigation on vitreousness

The yield of wet gluten increases with an increase in the amount of water in the irrigation rates (Figure 2). The increase is linear, and the resulting equation is provided with a sufficiently high coefficient of determinant. The exponential growth of the index with increasing irrigation rate is shown in the graph, the resulting equation is highly deterministic and it can be seen that it is between 25-30 mm for both varieties.

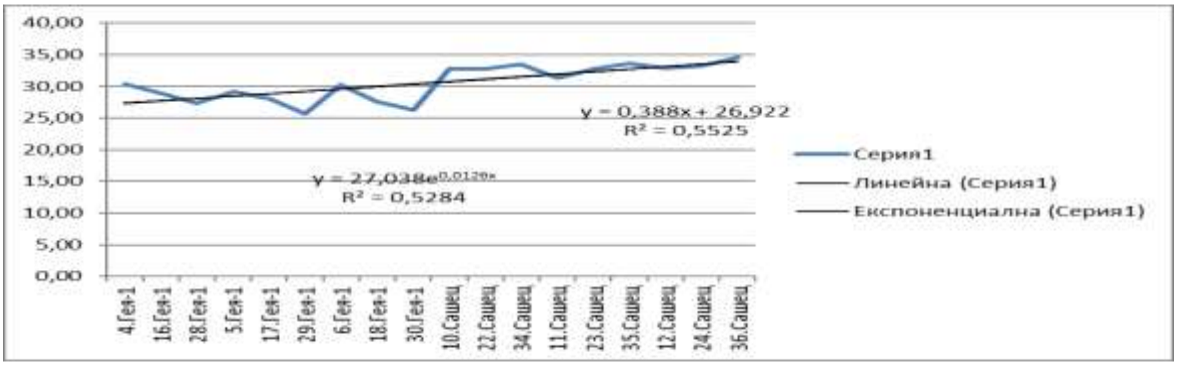


Figure 2. Effect of irrigation on wet gluten yield

Gluten release decreases with an increase in the amount of water and this decrease has a linear character with a sufficiently high degree of the determinant (Figure 3). The resulting exponential equation from the empirical data shows that the increase in the irrigation rate above 10 mm leads to a decrease in trait with the assurance of the equation with a high coefficient of the determinant.

Figure 3. Effect of irrigation on gluten release

The bakery power number is an trait that increases linearly with an increase in the amount of irrigation water (Figure 4). Empirical data show that the resulting equation has a high degree of the determinant and is sufficiently assured. The exponential curve shows that the highest values of this trait can be expected at 10 and 15 mm of water in the irrigation rate. The resulting equation has a high coefficient of determination

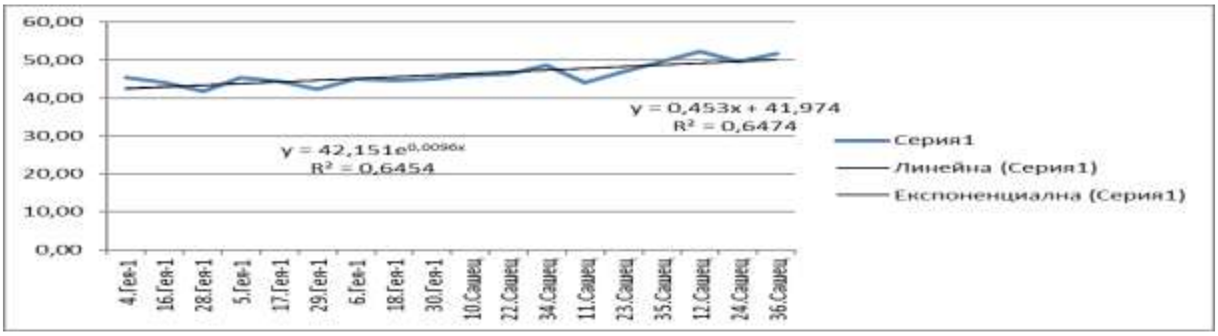


Figure 4. Effect of irrigation on bakery power number

The dry gluten also increased linearly with increasing amount of water used in both cultivars involved in the test (Figure 5). The linear equation derived from the empirical data has a high coefficient of determination. From the resulting exponential curve, we can conclude that the highest values of the trait are obtained when using an irrigation rate in the range of 10-20 mm.

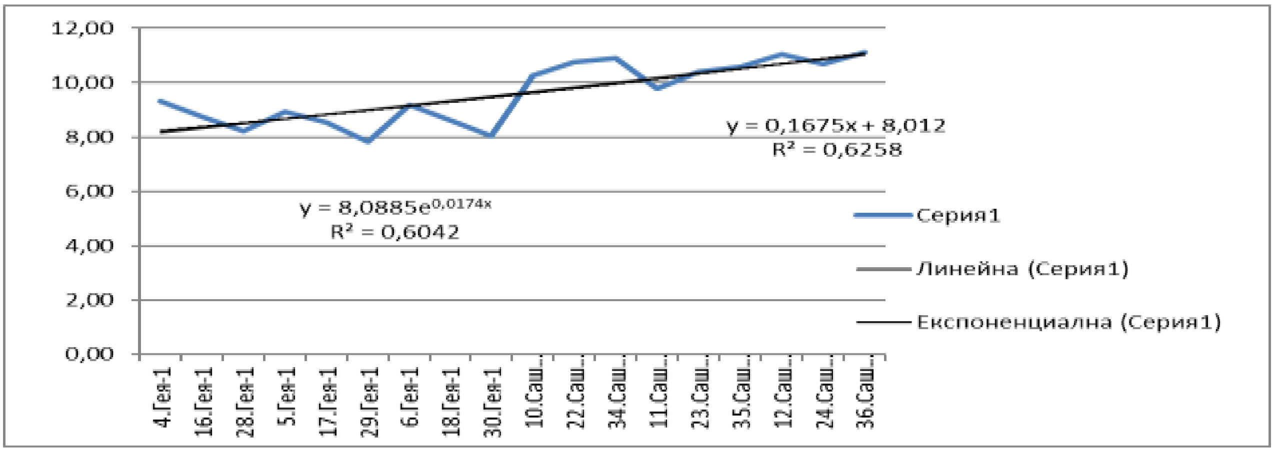


Figure 5 Effect of irrigation on dry gluten

Table 4 shows the influence of the genotype, the conditions of the year and the interaction between them on the studied traits. From the data in the table, it is clear that the interaction between genotype and environmental conditions has the highest influence on vitreousness, the genotype is second in importance. The genotype has the strongest influence on wet gluten yield, and the environmental conditions and the interaction between the two significant factors are equal. Gluten release has influence from the interaction conditions of year and genotype.

Table 4. ANOVA - Influence of the sources of variation on the studied traits

Trait	Sources of variation	SS	df	MS	F exp.	F tab.	ŋ,%	Sig.
Vitreousness	Genotype, Factor - A	3566.4	17	209.8	276.3	2.7	43.2	***
	Environment, Factor - B	261.4	2	130.7	172.2	7.4	3.2	***
	Interaction, A x B	4342.6	34	127.7	168.2	2.2	52.6	***
	Error	82.0	108	0.8			1.0	
	Total	8252.4	161				100.0	
Yield wet gluten	Genotype, Factor - A	1188.2	17	69.9	3216.8	2.7	45.7	***
	Environment, Factor - B	777.1	2	388.6	17883.5	7.4	29.9	***
	Interaction, A x B	634.3	34	18.7	858.6	2.2	24.4	***
	Error	2.3	108	0.02			0.1	
	Total	2602.0	161				100.0	
Gluten release	Genotype, Factor - A	781.6	17	46.0	154.4	2.7	16.8	***
	Environment, Factor - B	1814.4	2	907.2	3046.0	7.4	39.0	***
	Interaction, A x B	2021.0	34	59.4	199.6	2.2	43.5	***
	Error	32.2	108	0.3			0.7	
	Total	4649.1	161				100.0	
Bakery power number	Genotype, Factor - A	1382.5	17	81.3	99.8	2.7	21.4	***
	Environment, Factor - B	2521.3	2	1260.7	1547.2	7.4	39.0	***
	Interaction, A x B	2476.7	34	72.8	89.4	2.2	38.3	***
	Error	88.0	108	0.8			1.4	
	Total	6468.5	161				100.0	
Dry gluten	Genotype, Factor - A	195.4	17	11.5	1064.2	2.7	54.9	
	Environment, Factor - B	105.3	2	52.7	4875.1	7.4	29.6	
	Interaction, A x B	54.2	34	1.6	147.6	2.2	15.2	
	Error	1.2	108	0.0			0.3	
	Total	356.1	161				100.0	

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; F tab. - F tabular; ŋ - force of influence of the factor (%); *** - significant at $\alpha = 0.001$

CONCLUSIONS

1. Average variation in the varieties Geya1 and Sachets in the variants of fertilizer application and irrigation rates based on the coefficients of variation was found for vitreousness, dry gluten and gluten release, and it was weak in the yield of wet gluten and number of bakery strength.

2. The influence of the genotype factor is decisive for the yield of wet gluten, and the interaction between genotype and weather conditions for the vitreousness and the gluten release

3. The influence of the investigated factors and their interaction on the dry gluten trait has not been proven.

4. The established optimal number of application of the fertilizer rate by phases and the amount of water for irrigation for each studied trait will be included in an algorithm of

the system for intelligent management of agricultural processes in the cultivation of common winter wheat.

ACKNOWLEDGEMENT

This work is supported by the Bulgarian Ministry of Education and Science under the National Research Program "Smart crop production", Grant Д01-65/19.03.2021, approved by Decision of the Ministry Council No 866/ 26.11.2020

REFERENCES

Allami, H. A., M. Tabasizadeh, A. Rohani, H. Nayebzadeh, A. Farzad, 2020 Effect of ultrasonic irradiation on the properties and performance of biodiesel produced from date seed oil used in the diesel engine. Ultrason. Sonochem. 60, 104672.

Altenbach, S. B., 2012. New insights into the effects of high temperature, drought and post-anthesis fertilizer on wheat grain development. J. Cereal Sci. 56, 39–50.

Amirahmadi, E., M. Ghorbani, J. Moudr, J. Bernas, C. E. Mukosha, T. N. Hoang, 2024. Environmental Assessment of Dryland and Irrigated Winter Wheat Cultivation under Compost Fertilization Strategies Plants 13(4), 509. https://doi.org/10.3390/plants13040509.

Angelova T., E. Dimitrov, Zl. Uhr, G. Vida, B. Bozadzhiev, 2023. Evaluation of Hungarian common winter wheat varieties in terms of yield and technological qualities in the conditions of Central Southern Bulgaria. Research Institute of Mountain Stockbreeding and Agriculture, Troyan, Journal of Mountain Agriculture on the Balkans, 26 (2), 181-203.

Behnassi, M.; M. ElHaiba, 2022. Implications of the Russia–Ukrainewar for global food security. Nat. Hum. Behav. 6, 754–755.

Belcheva, L., 2018. National Grain Service 2018. https://sinor.bg/mb/1098- news-review.html Sinor. (BG)

Bielski, S.; R. Marks-Bielska, A. Zieli´nska-Chmielewska, K. Romaneckas, E. Šarauskis, 2021. Importance of agriculture in creating energy security—A case study of Poland. Energies 14, 2465.

Bouazzama, B., M. Boutfirass, A. Bahri, H. Lehamaoui, 2024. Effect of supplemental irrigation on yield and water productivity of durum wheat cultivated under drip irrigation in Tadla, Morocco. Moroccan Journal of Agricultural Sciences, 5(1).

Brankovic, G., D. Dodig, M. Zori´c, G. Surlan-Momirovic, V. Dragicevic and N. Djuric, 2014. Effects of climatic factors on grain vitreousness stability and heritability in durum wheat. Turk. J. Agric. For., 38, 429-440.

Brankovic, G., D. Dodig, M. Zori´c, G. Surlan-Momirovic, V. Dragicevic, N. Djuric, 2014. Effects of climatic factors on grain vitreousness stability and heritability in durum wheat. Turk. J. Agric. For., 38, 429-440.

Cacak-Pietrzak, G. 2008. The use of wheat in various branches of the food industry—Technological requirements. Prz. Zbo˙z. Młyn, 52, 11–13 (In Polish).

Cacak-Pietrzak, G., 2011. Studies on the effect of organic and conventional 200 system of plant production on the technological value of selected varieties of winter wheat. In Treatises and Monographs; Publications of Warsaw University of Life Sciences: Warsaw, Poland, pp. 1–83.

Campesi, G. P., A. Deligios, L. Ledda, F. Madau, G. Piluzza, G.A. Re. Sanna, F. L. Sulas, 2023. Starter irrigation in sulla as a promising practice to climate change adaptation of Mediterranean rainfed forage systems. Int. Agrophys. 2023, 37, 159–169.

Dalin, C., Y. Wada, T. Kastner, M. J. Puma, 2017. Groundwater depletion embedded in international food trade. Nature 543, 700–704.

de Oliveira Silva, A., G. A. Slafer, A. K. Fritz, R. P. Lollato, 2020b. Physiological basis of genotypic response to management in dryland wheat. Front. Plant Sci. 10, 1644.

Dilmurodovich, D. S., B. N. Bekmurodovich, K. N. Shakirjonovich, 2022. Creation of new drought-resistant, high-yielding and highquality varieties of bread wheat for rainfed areas. Br. J. Glob. Ecol. Sustain. Dev. 2, 61–73.

Ding, H., D. Liu, X. Lui, Y. Li, J. Kang, J. Lv, G. Wang, 2018. Photosynthetic and stomatal traits of spike and flag leaf of winter wheat (*Triticum aestivum* L.) under water deficit. Photosynthetica 56, 687–697.

Dizlek, H., A. L. Girard, J. M. Awika, 2022. High protein and gliadin content improves tortilla quality of a weak gluten wheat. LWT , 160, 113320. [CrossRef]

Erenstein, O.; M. Jaleta, K. A. Mottaleb, K. Sonder, J. Donovan, H. J. Braun, 2022. Global Trends in Wheat Production, Consumption and Trade. In Wheat Improvement; Reynolds, M.P., Braun, H.J., Eds.; Springer: Cham, Switzerland.

Eurostat. Performance of the Agricultural Sector, 2023. Available online: https://ec.europa.eu/eurostat/statistics-explained/.

Filipov, H., 2004. Evaluation of the quality of wheat by the appearance of the grain, Academic edition of the Agricultural University of Plovdiv, pp 60, (Bg).

Frenken, K., V. Gillet, 2012. Irrigation Water Requirement and Water Withdrawal by Country. Food and Agriculture Organization, United Nations.

Guerrini, L.; M. Napoli, M. Mancini, P. Masella, A. Cappelli, A. Parenti, S. Orlandini, 2020. Wheat grain composition, dough rheology and bread quality as affected by nitrogen and sulfur fertilization and seeding density. Agronomy, 10, 233.

Hamani, A. K. M., S. A. Abubakar, Y. Fu, D. F. Kpalari, G. Wang, A. Duan, X. Ju, 2024. The coupled effects of various irrigation scheduling and split nitrogen fertilization modes on post-anthesis grain weight variation, yield, and grain quality of drip-irrigated winter wheat (*Triticum aestivum* L.) in the North China Plain. Journal of Integrative Agriculture.

Hao, T., R. J. Jia. C Chen, Y. W. Du, Li. Zhao, H. Duan. 2023. Enhancing wheat gluten content and processing quality: An analysis of drip irrigation nitrogen frequency.Plants, 12 (23).

Hao, T.; Z. Zhu, Y. Zhang, S. Liu, Y. Xu, X. Xu, C. Zhao, 2023. Effects of Drip Irrigation and Fertilization Frequency on Yield,Water and Nitrogen Use Efficiency of Medium and Strong Gluten Wheat in the Huang-Huai-Hai Plain of China. Agronomy, 13, 1564. https:// doi.org/10.3390/agronomy13061564.

Hoff, H., 2018. Mainstreaming climate adaption into watershed management. Climate protection programme for developing countries. Indo-German Bilateral Project on Watershed Manage¬ment, 151p.

Hristov, J., 1967. Quality of the grain and flour of the wheat grown in ourcountry, Academy of Agricultural Sciences, Sofia-1967, Publishing House of theBulgarian Academy of Sciences (Bg).

Ivanova, D., H. Kirchev, 2018. Quality of common wheat varieties with different origin, grownin the ecological conditions of mizia. Agraren Universitet Plovdiv-Nauchni Trudove/Scientific Works of the Agrarian University-Plovdiv, 61(1), 13-18. DOI: 10.22620/sciworks.2018.01.002, (Bg).

Jankowski, K.J., L. Kijewski, B. Dubis.2015. Milling quality and flour strength of the grain of winter wheat grown in monoculture. Rom. Agric. Res. 32, 191–200.

Karadjov, G., R. Vasileva, M. Nikolova. 1998. Technology of bread, bakery and confectionery products. S., Zemizdat. (BG)

Khalid, A.; A. Hameed, M. F. Tahir, 2023. Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality. Front. Nutr. 10, 1053196.

Kumar, A., R. Nayak, S.R. Purohit, P.S. Rao. 2021. Impact of UV-C irradiation on solubility of Osborne protein fractions in wheat flour. Food Hydrocoll. 110, 105845. [CrossRef]

Lachutta, K., K. J. Jankowski, 2024. An Agronomic Efficiency Analysis of Winter Wheat at Different Sowing Strategies and Nitrogen Fertilizer Rates: A Case Study in Northeastern Poland. Agriculture 2024, 14, 442. https://doi.org/10.3390/ agriculture14030442.

Li, J. P., Y. Q. Wang, M. Zhang, Y. Liu, X. X. Xu, G. Lin, Z. M. Wang, Y. M. Yang, Y. H. Zhang, 2019b. Optimized micro-sprinkling irrigation scheduling improves grain yield by increasing the uptake and utilization of water and nitrogen during grain filling in winter wheat. Agric. Water Manag. 211, 59–69. https://doi.org/ 10.1016/j.agwat.2018.09.047.

Li, J., X. Liu, X. Yang, Y. Li, C. Wang, D. He, 2018. Proteomic analysis of the impacts of powdery mildew on wheat grain. Food Chem. 261, 30–35.

Li, M., Q. Yue, C. Liu, X. Zheng, J. Hong, N. Wang, K. Bian. 2021. Interaction between gliadin/glutenin and starch granules in doughduring mixing. LWT 148, 111624.

Li, Y. Q., R. J., Zhu, J. C. Tian, 2008. Influence of wheat protein contents and fractions on dough rheological properties as determined by using a reconstitution method. Agric. Sci. China 7, 395–404.

Liu, J. X., J. M. Zhang, G. R. Zhu, D. Zhu, Y. M. Yan, 2022. Effects of water deficit and high N fertilization on wheat storage protein synthesis, gluten secondary structure, and breadmaking quality. Crop J. 2022, 10, 216–223.

Meng, W. W., Z. W. Yu, Y. L. Zhang, Y. Shi, D. Wang, 2015. Effects of supplemental irrigation on water consumption characteristics and grain yield in different wheat cultivars. Chil. J. Agric. Res. 75, 216–223. https://doi.org/10.4067/s0718-58392015000200011.

Mitura, K., G. Cacak-Pietrzak, B. Feledyn-Szewczyk, T. Szablewski, M. Studnicki. 2023. Yield and grain quality of common wheat (*Triticum aestivum* L.) depending on the different farming systems (organic vs. integrated vs. conventional). Plants 12, 1022.

Mottaleb, K. A., K. Gideon, S. Sieglinde. 2022. Potential impacts of Ukraine-Russia armed conflict on global wheat food security: A quantitative exploration. Globa. Food Sec. 35, 100659.

Nenova, L., S. Stoyanova. 2011. Influence of systemic mineral fertilization on the yield and quality of winter soft wheat variety "Aglika" grown in conditions of highly leached chernozem, Bulletin of the Union of Scientists - Ruse • Series 3 • Agricultural and Veterinary Sciences, 11 -15. (BG)

Pawlak, K., M. Kołodziejczak, 2020. The role of agriculture in ensuring food security in developing countries: Considerations in the context of the problem of sustainable food production. Sustainability 12, 5488.

Peng, S. B., B. Bouman, R. M. Visperas, A. Castañeda, L. X. Nie, H. K. Park, 2006. Comparison between aerobic and flooded rice in the tropics: Agronomic performance in an eight-season experiment. Field Crops Res. 96, 252–259.

Qu,W. K., X. X. Xu, T. J. Hao, S. Liu, J. K. Zhao, F. G. Meng, C. X. Zhao, 2022. Effects of nitrogen application rate on annual yield and n-use efficiency of winter wheat-summer maize rotation under drip irrigation. J. Plant Nutr. Fert. 28, 1271–1282.

Rasheed, A., X. Xia, Y. Yan, R. Appels, T. Mahmood, Z. He, 2014. Wheat seed storage proteins: Advances in molecular genetics, diversity and breeding applications. J. Cereal Sci. 60, 11–24.

Reinhardt, J., P. Hilgert, M. Von Cossel, 2022. Yield performance of dedicated industrial crops on low-temperature characterized marginal agricultural land in Europe—A review. Biofuels Bioprod. Bioref. 16, 609–622.

Shahdany, S. M. H, A. Firoozfar, J. M. Maestre, I. Mallakpour, S. Taghvaeian, P. Karimi, 2018. Operational performance improvements in irrigation canals to overcome groundwater overexploitation. Agric. Water Manag. 204, 234–246.

Shewry, P. R. 2009. Wheat. Journal of Experimental Botany, Volume 60, Issue 6, April 2009, Pages 1537–1553, https://doi.org/10.1093/jxb/erp058.

Sokolenko, N. I., N. A., Galushko, N. M. Komarov, 2021. Sources of high-quality grain in winter common wheat breeding // Taurida Herald of the Agrarian Sciences, No. 3(27), 164–171. DOI: 10.33952/2542-0720-2021-3-27-164-171.

Stepniewska, S. 2016. Milling value of grain of selected wheat cultivars from crop years 2012–2014. Acta Agrophysica 23, 105–117.

Studnicki, M. 2023. Yield and Grain Quality of Common Wheat (*Triticum aestivum* L.) Depending on the Different Farming Systems (Organic vs. Integrated vs.Conventional), 12, 1022. https://doi.org/10.3390/.

Sun, Z. C., Y. H. Zhang, Z. Zhang, Y. M. Gao, Y. M. Yang, M. K. Han, Z. M. Wang, 2019. Significance of disposable presowing irrigation in wheat in increasing water use efficiency and maintaining high yield under winter wheat-summer maize rotation in the North China Plain. Agric. Water Manag, 225, 105766 https://doi.org/10.1016/j.agwat.2019.105766.

Van Der Broght, A., H. Goesaert, W. Veraverbke, J. Delcour, 2005.Fractionation of wheat and wheat flour into starch and gluten: overview of the mainprocesses and the factors involved. Journal of Cereal Science, 41, 221-237.

Wang, J., S. Gong, D. Xu, Y. Yu, Y. Zhao. 2013. Impact of drip and level-basin irrigation on growth and yield of winter wheat in the North China Plain. Irrigation Science, 31, 1025-1037.

Wieser, H. 2007. Chemistry of gluten proteins. Food Microbiol. 24, 115–119.

Wieser, H., R. Kieffer. 2001. Correlations of the amount of gluten protein types to the technological properties of wheat flours determined on a micro-scale. J. Cereal Sci. 2001, 34, 19–27.

Yao, C., J. Li, Z. Zhang, Y. Liu, Z. Wang, Z. Sun, Y. Zhang, 2023. Improving wheat yield, quality and resource utilization efficiency through nitrogen management based on micro-sprinkler irrigation. Agricultural Water Management, 282, 108277.

Zain, M., Z. Si, H. Ma, M. Cheng, A. Khan, F. Mehmood, A. Duan, C. Sun, 2023. Developing a tactical irrigation and nitrogen fertilizer management strategy for winter wheat through drip irrigation. Front. Plant Sci. 14:1231294. doi: 10.3389/fpls.2023.1231294.

Zhang, H. B., K. Gu, S. B. Han, D. Wang, 2019. Effects of supplemental irrigation on the accumulation, distribution and transportation of 13C-photosynthate, yield and water use efficiency of winter wheat. Agric. Water Manag 214, 1–8.

Zhang, P., Z. He, D. Chen, Y. Zhanga, O. R. Larroquee, X. Xia, 2007. Contribution of common wheat protein fractions to dough properties and quality of northern-style Chinese steamed bread. J. Cereal Sci. 46, 1–10.

Zhang, X., Y. Wang, H. Sun, S. Chen, L. Shao, 2013. Optimizing the yield of winter wheat by regulating water consumption during vegetative and reproductive stages under limited water supply. Irrigation Science, 31, 1103-1112.

Zhang, Y., M., Xu, X. Zhang, Y. Hu, G. Luan, 2022. Application of zein in gluten-free foods: A comprehensive review. Food Res. Int. 160, 111722.

Zhen, S., X. Deng, X. Xu, N. Liu, D. Zhu, Z. Wang, Y. Yan, 2020. Effect of high-nitrogen fertilizer on gliadin and glutenin subproteomes during kernel development in wheat (*Triticum aestivum* L.). Crop J. 2020, 8, 38–52.

Zingale, S., P. Guarnaccia, A. Matarazzo, G. Lagioia, C. Ingrao, 2022. A Systematic Literature Review of Life Cycle Assessments in the Durum Wheat Sector. Sci. Total Environ, 844, 157230.

Zlateva, D., 2010. Investigation of the yield and physical properties of gluten from wheat flour. Izvestia, edition of the University of Economics–Varna, 3.

Zubair, M., L. Akhtar, R. Minhas, M. Bukhari, S. Hussain, I. Ali, M. T. Mahmood, M. Rehman, A. M. Imran, R. Ullah, 2021. Assessment of Wheat Genotypes for Quality Attributes Grown under Irrigated and Rainfed Conditions. Egyptian Journal of Agronomy, 43(1), 97-104.

STABILITY OF QUALITY TRAITS IN COMMON WINTER WHEAT GENOTYPES GROWN UNDER DIFFERENT NUTRITIONAL AND IRRIGATION REGIMES FOR INCLUSION IN AN ALGORITHM OF THE SYSTEM FOR INTELLIGENT MANAGEMENT OF AGRICULTURAL PROCESSES

Evgeniy DIMITROV, Stanislav STAMATOV, Teodora ANGELOVA, Zlatina UHR

IPGR "K. Malkov", Sadovo, Agricultural Academy, Bulgaria

ABSTRACT

In the experimental field of the IPGR, Sadovo town. A three-year experiment with two varieties of common winter wheat grown under different nutritional and irrigation regimes was conducted in the field of IPGR, Sadovo, in the period 2021-2023. Eighteen fertilizing treatments were used in the experiment including single, double and triple application of nitrogen fertilizer (17 kg N) in combination with different irrigation rates of 10, 20 and 30 mm. The fertilizer and irrigation rates were applied at the tillering, end-tillage beginning-tillage and tillering phases. Grain vitreousness, wet gluten yield, gluten release, baking strength number and dry gluten were used to assess grain quality. The stability of the studied traits of two wheat cultivars, Geya 1 and Sashets, was evaluated by the stability variants σi^2 and Si^2 according to Shukla (1972), the ecovalance Wi according to Wricke (1962) and the phenotypic stability criterion (Ysi) according to Kang (1993). IPCSSVKYSI (Interactive program for calculating Shukla's stability index (Ysi)) developed by Kang and Magari (1995) was used to determine the stability index. The most stable value for the parameters vitreousness, gluten release and dry gluten was characterized by the cultivar Sashetz under the variation with double fertilizer application rate and irrigation rate of 10 mm. Stability of the trait baking strength number was reported in Geya 1 in combination with double nitrogen application and minimum irrigation. Cultivar Sashets realized the highest stability of the trait wet gluten yield in the variant with single fertilizer application and maximum irrigation rate. The obtained results are useful from the breeding point of view for increasing the stability of quality traits. The data obtained from the different nutrient regimes can be incorporated into the algorithm of the intelligent agricultural process control system to achieve a consistency in the values of the studied parameters.

Key words: Smart farming, Common winter wheat, Stability, Quality indicators

INTRODUCTION

In today's context of steadily increasing population and changing climate, increasing the yield and improving the quality of winter wheat grain is becoming increasingly important worldwide. This requires the introduction of varieties with high productivity and ecological plasticity to the environmental conditions in which they are grown. There is a need for in-depth research on the yield and quality potential of varieties under different agro-ecological conditions, so each element of wheat crop technology has been studied by a number of researchers (Sevov and Delibaltova, 2013; Kuneva et al., 2014; Barakova et al., 2018; Delchev and Angelova, 2019; Stoyanova et al., 2020; Van Frank et al.,2020; Minoli et al., 2022).

One of the main tasks of breeding programs is the introduction of varieties with high ecological plasticity, quality characteristics and with good stability varieties. Any variety can have high grain yield and high ecological plasticity, regardless of its quality potential (Tsenov and Atanasova, 2015; Stoyanova et al., 2020).

The problem of wheat grain quality is an integrative indicator of the interaction between the genotype of the variety, natural and ecological features, agrotechnical and organizational and economic conditions of cultivation (Rozbicki, 2014; Krystkowiak, 2016; Пахотина et al., 2020; Fomenko, 2018). It is considered that the leading factor influencing the quality of wheat grain is the hereditary characteristics of the variety (Смирнова, 2018).

The results show that the influence of genotype and its interaction with the surrounding environment has been demonstrated in all observed traits (Reckling et al., 2021; Dimitrov et al., 2023).

The increasing demand for high technological quality in wheat necessitates the need to increase protein and gluten content, improving dough strength and stability (Blandino et al., 2016).

As a consequence of global climate change, the sustainability of cropping systems in agriculture is decreasing. There is a decline in cereal production, which has also been reported for wheat in Europe (Kahiluoto, H., 2019; Kahiluoto et al., 2019; Piepho, 2019;).

As temperature increases, wheat yields are expected to decrease by 6% for every 1°C increase in temperature. Therefore, wheat grain yields must increase by 60% by 2050 to meet the needs of a growing world population (https://www.fao.org/worldfoodsituation/csdb/en; Asseng et al., 2015; Uhr et al., 2023). In this regard, the study of crop response to water deficit is important in areas where water resources are limited. Many of them in which wheat is grown are characterized by low humidity and insufficient rainfall (Bouazzama, 2024).

Wheat is one of the majornon-grain crops in irrigated farming systems (Frenken and Gillet, 2012; De Oliveira Silva et al., 2020b; Leghari et al.,2024).

Different primes are used for its irrigation. Conventional surface irrigation methods use large amounts of water, resulting in irrational use of limited water resources.

According to the literature, among all the irrigation methods used, drip irrigation is the most efficient and can be successfully practiced for irrigating a wide range of crops (Bouazzama, 2024). It potentially saves more than 20% of irrigation water compared to surface irrigation (Karrou et al., 2011)

Scarcity of water resources and unwise application of nitrogen fertilizers appear to be a problem in wheat production.(Yao, 2023).

Hence proper water management and use of nitrogen fertilizers by conducting studies on their application is necessary to achieve good productivity (Zain, 2023).

Yao et al. (2023) investigated the application of irrigation and nitrogen nutrition regimen to simultaneously improve wheat grain yield, its quality, water and nitrogen use efficiency and clarify its improvement mechanism. The results showed that increasing the nitrogen fertilization ratio significantly improved the chlorophyll content, net photosynthetic rate, glutamine synthetase, nitrate reductase activity and soluble protein content during grain pouring (Yao et al., 2023).

It has been found that, unwise use of nitrogen fertilizers leads to environmental problems such as water eutrophication, nitrate contamination in groundwater, greenhouse gas emissions etc (Axinte et al., 2015; Tedone et al., 2018).

Because of this obstacle, proper nitrogen fertilizer management is very important for high yield, high quality and environmentally friendly production of common winter wheat. Scientists devise the most effective N fertilizer management methods by separating the application of N fertilizer in different crop development phases,as well as changing the form of N fertilizer itself and so on (Shi et al., 2012; Li et al., 2018; Zhang et al., 2021; Wan et al., 2021; Lyu et al., 2022).

Nitrogen is an important factor influencing the protein and wet gluten content of wheat (Blandino, 2016), and the quality of wheat dough and pasta is largely influenced by the protein content and gluten quality Gluten is a protein that has elastic and plastic properties after hydration and mixing by hand or machine (Edwards, 1999; Morris, 2016; Zubair, 2021; Trevisan, 2022).

Stability is the ability of varieties to express their genetic potential under a wide range of growing conditions (Ann icchiarico, 2002). Stable expression of varieties and their wide adaptation in terms of yield and quality traits is the goal of many breeding programs (Atanasova et al., 2008; Aminzadeh, 2010; Parveen et al., 2010, Stoyanova et al., 2020; Bondarenko and Nazarenko, 2020; Uhr et al., 2021).

Different parameters are known to assess phenotypic stability (Eberhard and Russell, 1966; Shukla, 1972), but the most reliable method for simultaneous stability assessment is Kang's (1993) Ysi parameter.

Through this criterion, the value of each variety is presented, taking into account both the value of the indicator and the stability of the variant. The value of this criterion is that, using non-parametric methods and statistical evidence of differences, a summary score is obtained, ranking the variants according to their economic value.

Irrigation methods and nitrogen (N) fertilization regimes have complex effects on wheat physiology, growth, and development, resulting in regulation of wheat grain yield and quality.

The purpose of the study is the selection of an effective nutritional regimen to achieve high and stable results of the studied traits, as the data obtained from the study will be included in an algorithm of the system for intelligent management of agricultural processes.

MATERIAL AND METHOD

The experiment was carried out in the breeding field of IRGR, Sofia. Sadovo in the period 2021-2023. Bulgarian varieties Geya 1 - medium strength wheat (Group B) and Sashetz - strong wheat (Group B) were used in the experiment.

The response of grain vitreousness, % (BDS 13378:1976), wet gluten yield, % (BDS EN ISO 21415-1:2007), gluten relaxation, mm (BDS 13375:1990/Amendment 1:1993), baking strength number, AU (BDS 13375:1990/Amendment 1:1993) and dry gluten (%) BDS EN ISO 21415-4:2007 was monitored.

The technology under which the varieties were grown consisted of eighteen feeding treatments involving single, double and triple application of the nitrogen fertiliser rate (17 kg N) in the following scheme:

-The total amount applied at the tillering-early spindling stage.

-½ of the amount applied in the tillering phase-early stem elongation phase and ½ applied in the stem elongation phase.

-1/3 of the quantity imported in the tillering phase - start of stem elongation, 1/3 imported in the stem elongation phase and 1/3 imported in the heading phase.

Application is in combination with different irrigation rates of 10, 20 and 30 mm. The irrigation rates were applied in the tillering, end-till-early tillering and stem elongation phases.

The mathematical processing of the data was performed using Microsoft Excel for Windows.

RESULTS AND DISCUSSION

The results of the qualitative indices studied and their stability under different diets are presented in Tables 1, 2, 3 and 4. It is evident from the data that the highest value of the trait vitreousness (Table 1) during the study period was obtained in cultivars Geya 1 and Sashchets, variant №1 and variant №10. In variant №1, the nitrogen fertilizer rate was applied once in combination with an irrigation rate of 10 mm, while variant №10 included a double fertilizer application and an irrigation rate of 20 mm. The lowest value of the trait vitreousness is characterised by the variety Sashetz (№6), where the nitrogen fertiliser rate was applied once in combination with the maximum irrigation rate.

Regarding the stability of the studied parameters, genotypes showing lower values under the different diets are estimated to be more stable because they interact less with the environmental conditions. Negative values of the σi^2 and Si^2 indicators are assumed to be 0. For reliably high values of either σi^2 or Si^2, genotypes are considered unstable. In Wricke's equilibrium Wi, the higher the parameter values at different feeding levels, the lower their stability. In our study, the results showed that the highest vitreous stability (Table 1) was reported for variant 8 (two nitrogen and 20 mm water application) and variant 2 (two nitrogen and 30 mm water application). In these variants, the differences in vitreous values between years were smaller. As not stable, it can be pointed out that feeding variants

№9 and №14 show the highest variation between the values of the indicator during the years of the study

Very useful information on the value of genotypes is provided by Kang's YSi indicator for simultaneous assessment of qualitative indicators and their stability, the indicator being based on the reliability of differences between different values and the variance of interaction with the environment. The value of this criterion is that using non-parametric methods and statistical evidence of differences, we obtain a summary score ranking genotypes in descending order of their economic value. In our study, the most valuable varieties were Geya 1 (variant №12) and Sashez (variant №18) in which nitrogen fertilizer was applied twice (variant №12) and three times (variant №12) with a maximum irrigation rate of 30 mm.

Table 1. Vitreousness for the period 2021-2023

Variant, №	Variety	Value				Stability parameters				Fertilizing			Irrigation rate, mm
		2021	2022	2023	X	σ^2_i	s^2_i	W_i^2	YS i	N, g	N, g	N, g	
1	Geya	50	48	56	51.	22.8	8.4	176.9	11	50	-	-	10
2	Sachet	32	50	40	40.	49.1	17.7	363.9	31	50	-	-	10
3	Geya	44	42	48	44.	14.5	4.1	117.7	14	50	-	-	20
4	Sachet	41	42	48	43.	17.0	11.2	135.3	17	50	-	-	20
5	Geya	42	48	34	41.	26.9	14.1	205.6	27	50	-	-	30
6	Sachet	28	36	38	34.	19.5	21.8	152.8	26	50	-	-	30
7	Geya	54	44	44	47.	36.5	22.5	274.3	19	25	25	-	10
8	Sachet	44	43	40	42.	2.6	4.0	33.3	15	25	25	-	10
9	Geya	28	28	50	35.	153.	103.	1105.	35	25	25	-	20
10	Sachet	54	56	44	51.	24.6	21.1	189.4	11	25	25	-	20
11	Geya	48	43	40	43.	15.6	14.4	125.3	6	25	25	-	30
12	Sachet	40	47	44	43.	3.5	4.0	39.4	2	25	25	-	30
13	Geya	54	50	40	48.	39.5	42.1	295.3	18	16	16	16	10
14	Sachet	54	43	38	45.	61.2	56.0	449.5	25	16	16	16	10
15	Geya	52	55	46	51.	9.3	8.4	80.8	6	16	16	16	20
16	Sachet	44	53	40	45.	21.2	4.8	164.9	16	16	16	16	20
17	Geya	38	49	44	43.	14.3	8.8	116.4	4	16	16	16	30
18	Sachet	38	56	50	48.	53.2	32.9	393.0	16	16	16	16	30
Mean		43.6	46.3	43.6	44.								
Minimum		28	28	34	34.								
Maximum		54	56	56	51.								
Standard deviation					4.8								
Coefficient of variation					10.								
Standard error of mean					1.1								

σ^2_i – Shukla's stability variance; s^2_i – deviation from redression; W_i^2 – Wricke's ecovalence; YSi – Kang's rank-sum;

The highest values of wet gluten yield (Table 2) was recorded in variants 18 and 15 in which there was a triple nitrogen application with 30 and 20 mm amount of water. The lowest result was recorded in variety Sashetz (variant № 6) involving a single application of fertilizer and an irrigation rate of 30 mm.

The calculated stability indices of wet gluten yield show us that high stability was recorded in feeding variants №5 and №8, while low stability over the years was present in feeding regimes №1 and №11. According to Kang's YSi index, we can assume that the variant №18 is evaluated as the most efficient since it showed high yield wet gluten values in all three years of the study.

Table 2. Wet gluten yield for the period 2021-2023

Variant, №	Variety	Value				Stability parameters				Fertilizing			Irrigation rate, mm
		2021	2022	2023	X	σ^2_i	s^2_i	W_i^2	YSi	N, g	N, g	N, g	
1	Geya 1	32.4	25.9	32.8	30.	14.	12.	107.	28	50	-	-	10
2	Sachet	30.5	27.8	28.4	28.	1.9	0.4	15.3	18	50	-	-	10
3	Geya 1	27.8	26.4	28.0	27.	5.9	0.7	43.9	27	50	-	-	20
4	Sachet	31.2	25.8	30.3	29.	7.4	5.9	55.1	28	50	-	-	20
5	Geya 1	31.6	26.7	26.0	28.	0.0	0.2	1.9	15	50	-	-	30
6	Sachet	27.8	27.0	22.4	25.	2.4	2.6	18.8	25	50	-	-	30
7	Geya 1	33.6	27.7	29.2	30.	1.6	1.9	13.5	15	25	25	-	10
8	Sachet	28.9	26.8	27.2	27.	2.3	0.2	18.2	21	25	25	-	10
9	Geya 1	26.2	28.0	24.8	26.	6.5	2.1	48.1	32	25	25	-	20
10	Sachet	38.8	30.5	28.8	32.	5.9	0.3	44.0	20	25	25	-	20
11	Geya 1	39.0	32.5	26.8	32.	10.	1.5	74.9	23	25	25	-	30
12	Sachet	34.9	35.9	29.6	33.	6.2	6.2	46.2	16	25	25	-	30
13	Geya 1	36.6	30.7	26.6	31.	4.5	0.5	33.8	19	16	16	16	10
14	Sachet	38.3	32.3	27.5	32.	6.4	1.0	47.4	21	16	16	16	10
15	Geya 1	35.2	35.1	30.6	33.	3.0	2.9	23.7	10	16	16	16	20
16	Sachet	38.2	31.9	28.6	32.	3.7	0.1	28.3	14	16	16	16	20
17	Geya 1	37.5	31.6	30.5	33.	0.8	0.2	7.8	7	16	16	16	30
18	Sachet	36.3	34.2	33.2	34.	0.8	0.0	7.7	3	16	16	16	30
Mean		33.6	29.8	28.4	30.								
Minimum		26.2	25.8	22.4	25.								
Maximum		39.0	35.9	33.2	34.								
Standard deviation					2.8								
Coefficient of variation					9.1								
Standard error of mean					0.7								

σ^2_i – Shukla's stability variance; s^2_i – deviation from redression; W_i^2 – Wricke's ecovalence; YSi – Kang's rank-sum;

The data obtained for gluten release (Table 3) show us that the most efficient regime is the one with a single application of the entire nitrogen rate with 20 mm of water (variant 4). Variant 16 was characterized as not effective and with the lowest value in the indicator of gluten release. High stability of the studied indicator was found in the fertilizing variants 8 and 12, and as not stable were evaluated the fertilizing №9. The most valuable according to the YSi index was variant №3, which showed a combination of high and relatively constant values of the studied indicator during the test period.

Table 3. Gluten release for the period 2021-2023

Variant, №	Variety	Value				Stability parameters				Fertilizing			Irrigation rate, mm
		2021	2022	2023	X	σ^2_i	s^2_i	W_i^2	YSi	N, g	N, g	N, g	
1	Geya 1	22.5	8.0	28.0	19.	32.	0.5	235.	16	50	-	-	10
2	Sachet	15.0	14.0	27.0	18.	17.	16.	133.	18	50	-	-	10
3	Geya 1	21.0	13.0	23.0	19.	0.9	0.8	13.3	7	50	-	-	20
4	Sachet	25.5	8.0	25.0	19.	32.	10.	237.	16	50	-	-	20
5	Geya 1	15.5	13.0	24.0	17.	6.8	7.1	54.9	15	50	-	-	30
6	Sachet	20.5	10.5	26.5	19.	12.	0.3	97.0	14	50	-	-	30
7	Geya 1	19.0	11.0	18.0	16.	2.4	3.4	23.7	16	25	25	-	10
8	Sachet	15.5	12.0	19.0	15.	0.1	0.6	7.2	12	25	25	-	10
9	Geya 1	14.5	20.5	10.0	15.	72.	0.5	525.	31	25	25	-	20
10	Sachet	18.0	10.0	21.0	16.	1.6	0.5	18.2	14	25	25	-	20
11	Geya 1	18.0	12.0	15.0	15.	7.3	4.5	58.3	9	25	25	-	30
12	Sachet	17.5	11.0	14.0	14.	8.3	5.7	65.4	25	25	25	-	30
13	Geya 1	14.0	17.0	24.0	18.	17.	16.	127.	18	16	16	16	10
14	Sachet	15.5	11.0	21.0	15.	1.1	1.6	14.6	14	16	16	16	10
15	Geya 1	11.0	11.5	23.0	15.	18.	19.	138.	26	16	16	16	20
16	Sachet	15.0	8.0	15.2	12.	0.7	1.6	11.6	20	16	16	16	20
17	Geya 1	17.5	14.0	9.5	13.	37.	11.	275.	33	16	16	16	30
18	Sachet	15.5	9.0	15.0	13.	1.4	1.9	16.5	22	16	16	16	30
Mean		17.3	11.9	19.9	16.								
Minimum		11.0	8.0	9.5	12.								
Maximum		25.5	20.5	28.0	19.								
Standard deviation					2.3								
Coefficient of variation					13.								
Standard error of mean					0.5								

σ^2_i – Shukla's stability variance; s^2_i – deviation from redression; W_i^2 – Wricke's ecovalence; YSi – Kang's rank-sum;

For the baking strength number (Table 4), the values of the different options ranged from 41.7 (variant) to 52.3 (variant16). The calculated stability indices (σ^2_i, W_i^2) show us that the most stable values were obtained under a fertilazimg regime involving a double application of the fertilizer rate combined with an irrigation rate of 20 mm of water (variant 7). Low stability of the indicator was recorded in the cultivar Sachets involving a threefold application of the fertilizer rate and a maximum irrigation rate (variant 17). A combination of high and stable values according to the YSi index was obtained in variants 11 and 12, both involving a two-fold fertilizer application and an irrigation rate of 30 mm of water.

Table 4. Bakery strength number for the period 2021-2023

№	Variety	Value				Stability parameters				Fertilizing			Irrigation rate, mm
		2021	2022	2023	X	σ^2_i	s^2_i	W_i^2	YS_i	N, g	N, g	N, g	
1	Geya 1	40	56	40	45.3	17.3	3.8	131.5	22	500	-	-	10
2	Sachets	46	46	40	44.0	10.0	6.1	79.7	25	500	-	-	10
3	Geya 1	40	46	39	41.7	0.8	1.1	14.4	20	500	-	-	20
4	Sachets	40	56	40	45.3	17.2	3.6	130.8	12	500	-	-	20
5	Geya 1	46	47	40	44.3	7.8	5.9	63.9	22	500	-	-	30
6	Sachets	41	51	35	42.3	9.3	2.2	74.4	26	500	-	-	30
7	Geya 1	42	51	42	45.0	0.7	1.9	13.7	12	250	250	-	10
8	Sachets	45	48	41	44.7	3.1	2.3	30.6	17	250	250	-	10
9	Geya 1	45	41	49	45.0	63.6	1.9	460.6	17	250	250	-	20
10	Sachets	43	55	40	46.0	7.8	0.8	63.7	15	250	250	-	20
11	Geya 1	43	52	44	46.3	1.8	3.0	21.2	10	250	250	-	30
12	Sachets	44	55	47	48.7	5.3	6.6	46.1	10	250	250	-	30
13	Geya 1	48	44	40	44.0	25.5	13.6	189.9	14	166	166	166	10
14	Sachets	47	54	40	47.0	6.2	4.5	52.4	12	166	166	166	10
15	Geya 1	55	54	40	49.7	35.6	37.1	261.2	19	166	166	166	20
16	Sachets	47	65	45	52.3	32.6	2.5	240.4	16	166	166	166	20
17	Geya 1	44	48	57	49.7	79.6	31.7	574.6	18	166	166	166	30
18	Sachets	47	62	46	51.7	14.9	2.5	114.6	13	166	166	166	30
Mean		44.6	51.7	42.5	46.3								
Minimum		40	41	35	41.7								
Maximum		55	65	57	52.3								
Standard deviation					3.0								
Coefficient of variation					6.5								
Standard error of mean					0.7								

σ^2_i – Shukla's stability variance; s^2_i – deviation from redression; W_i^2 – Wricke's ecovalence; YSi – Kang's rank-sum;

The dry gluten values (Table 5) ranged from 7.8 (variant 6) to 11.1 (variant 18). In this indicator, the regularity was observed that the results obtained with a single application of nitrogen were lower compared to two and three applications of the fertilizer rate. The data obtained from the stability indices show us that the highest stability was obtained with variant №8, while the stability reported with variant No. 1 was poor. According to the YSi index as the most effective fertilazing regime we can determine variant №17, including triple application of nitrogen fertilizer and maximum irrigation rate.

Table 5. Dry gluten of winter common wheat for the period 2021-2023

№	Variety	Value				Stability parameters				Fertilizing			Irrigation rate, mm
		2021	2022	2023	X	σ^2_i	s^2_i	W_i^2	YS_i	N, g	N, g	N, g	
1	Geya	9.9	8.6	9.4	9.3	0.89	0.	6.	28	50	-	-	10
2	Sachet	9.2	8.6	8.4	8.7	0.28	0.	2.	19	50	-	-	10
3	Geya	8.5	8.4	7.7	8.2	0.30	0.	2.	23	50	-	-	20
4	Sachet	9.6	8.5	8.7	8.9	0.44	0.	3.	23	50	-	-	20
5	Geya	9.7	8.0	7.8	8.5	0.23	0.	1.	19	50	-	-	30
6	Sachet	8.4	8.4	6.7	7.8	0.12	0.	1.	21	50	-	-	30
7	Geya	10.7	8.7	8.2	9.2	0.26	0.	2.	16	25	25	-	10
8	Sachet	9.4	8.8	7.6	8.6	-	0.	0.	15	25	25	-	10
9	Geya	8.2	8.1	7.8	8.0	0.48	0.	3.	29	25	25	-	20
1	Sachet	12.1	10.1	8.5	10.	0.60	0.	4.	23	25	25	-	20
1	Geya	12.4	11.2	8.7	10.	0.65	0.	4.	21	25	25	-	30
1	Sachet	11.2	11.8	9.7	10.	0.44	0.	3.	13	25	25	-	30
1	Geya	11.5	10.0	7.9	9.8	0.58	0.	4.	23	16	16	16	10
1	Sachet	12.2	10.6	8.5	10.	0.61	0.	4.	23	16	16	16	10
1	Geya	11.4	11.6	8.7	10.	0.58	0.	4.	19	16	16	16	20
1	Sachet	11.9	11.8	9.4	11.	0.31	0.	2.	10	16	16	16	20
1	Geya	11.8	10.5	9.8	10.	0.04	0.	0.	7	16	16	16	30
1	Sachet	11.3	11.5	10.6	11.	0.39	0.	3.	10	16	16	16	30
Mean		10.5	9.7	8.6	9.6								
Minimum		8.2	8.0	6.7	7.8								
Maximum		12.4	11.8	10.6	11.								
Standard deviation					1.1								
Coefficient of variation					11.								
Standard error of mean					0.3								

σ^2_i – Shukla's stability variance; s^2_i – deviation from redression; W_i^2 – Wricke's ecovalence; YSi – Kang's rank-sum;

According to the calculated coefficients of variation of the qualitative indicators studied, we can summarize that the use of different fertilazing levels did not significantly affect the values obtained for the traits studied. For example, the variation in the number of baking strength and wet gluten yield was estimated to be low, with CV% values of 6.5 and 9.1%, respectively. Variation in vitreousness (10.9%), dry gluten (11.9%) and gluten release (13.8%) was moderate. Slight to moderate variation in qualitative traits has also been found in our previous studies (; Dimitrov et al., 2020; Uhr et al., 2020).

Table 6 presents the analysis of variance of the results, in which the influence of the factors genotype (factor A), growing conditions (factor B) and their interaction (AxB) on the studied quality indicators was evaluated.

Analysis of genotypex interaction is particularly important for the breeding process (Yan and Hunt, 2001). Very often, high stability of a given trait is associated with low levels of manifestation and vice versa (Tsenov et al., 2004; Atanasova et al., 2010).

The proven influence of the genotype x environment factors for all traits studied gives us reason to accept as reliable the data presented in the previous tables on the stability of genotypes grown at different levels of nutrition. From Table 6 it is evident that the interaction of the factors AxB, e had the strongest influence on vitreousness (52.6%) and gluten relaease (43.5%). The genotype factor was decisive in wet gluten yield (45.7%) and dry gluten, while the influence of different fertilazing levels was of primary importance on baking strength number (39.0%).

Table 6. ANOVA - Influence of the sources of variation on the studied traits

Trait	Sources of variation	SS	df	MS	F exp.	F tab.	ŋ,%	Sig.
Vitreousness	Genotype, Factor - A	3566.4	17	209.8	276.3	2.7	43.2	***
	Environment, Factor - B	261.4	2	130.7	172.2	7.4	3.2	***
	Interaction, A x B	4342.6	34	127.7	168.2	2.2	52.6	***
	Error	82.0	108	0.8			1.0	
	Total	8252.4	161				100.0	
Wet gluten yield	Genotype, Factor - A	1188.2	17	69.9	3216.8	2.7	45.7	***
	Environment, Factor - B	777.1	2	388.6	17883.5	7.4	29.9	***
	Interaction, A x B	634.3	34	18.7	858.6	2.2	24.4	***
	Error	2.3	108	0.02			0.1	
	Total	2602.0	161				100.0	
Gluten release	Genotype, Factor - A	781.6	17	46.0	154.4	2.7	16.8	***
	Environment, Factor - B	1814.4	2	907.2	3046.0	7.4	39.0	***
	Interaction, A x B	2021.0	34	59.4	199.6	2.2	43.5	***
	Error	32.2	108	0.3			0.7	
	Total	4649.1	161				100.0	
Bakery strength number	Genotype, Factor - A	1382.5	17	81.3	99.8	2.7	21.4	***
	Environment, Factor - B	2521.3	2	1260.7	1547.2	7.4	39.0	***
	Interaction, A x B	2476.7	34	72.8	89.4	2.2	38.3	***
	Error	88.0	108	0.8			1.4	
	Total	6468.5	161				100.0	
Dry gluten	Genotype, Factor - A	195.4	17	11.5	1064.2	2.7	54.9	
	Environment, Factor - B	105.3	2	52.7	4875.1	7.4	29.6	
	Interaction, A x B	54.2	34	1.6	147.6	2.2	15.2	
	Error	1.2	108	0.0			0.3	
	Total	356.1	161				100.0	

SS - sum of squares; gf - degrees of freedom; MS - variance; F exp. - F experimental; F tab. - F tabular; ŋ - force of influence of the factor (%); *** - significant at $\alpha = 0.001$

CONCLUSIONS

The highest stability of the quality parameters vitreousness, gluten release and dry gluten during the study period was realized by the variety Sashetz at two times application of the nitrogen fertilizer rate combined with an irrigation rate of 10 mm water. High stability of results in wet gluten yield was characterized by Geya 1 with a fertilazing regime involving the use of a single application of fertilizer and a maximum irrigation rate of 30 mm of water. In baking strength number, stability of results obtained was recorded in variety Geya 1 by using twice nitrogen rate and minimum water application.

A combination between high values and stability of the obtained results of quality parameters during each of the years of the study were obtained with the following diets:

- vitreousness, baking strength number: double application of nitrogen fertilizer with 10 mm irrigation rate
- wet gluten yield: three applications of nitrogen fertiliser at 30 mm irrigation rate
- gluten release: single application of nitrogen fertiliser at 20 mm irrigation rate

dry gluten: three applications of nitrogen fertiliser at 30 mm irrigation rate

The results of the research will help the breeding process to achieve stability and high values of the studied quality indicators by using effective nutritional regimes.

ACKNOWLEDGEMENT

This work is supported by the Bulgarian Ministry of Education and Science under the National Research Program “Smart crop production”, Grant Д01-65/19.03.2021, approved by Decision of the Ministry Council No 866/ 26.11.2020

REFERENCES

Aminzadeh, G. R., 2010. Evaluation of seed yield stability of wheat advanced genotypes in Ardabil, Iran. Research Journal of Environmental Science, 4(5), 478-482.

Annicchiarico, P., 2002. Genotype x environment interactions:challenges and opportunities for plant breeding and cultivar recommendations (No. 174), FAO Plant.

Asseng, S., M. Semenov, P. Stratonovitch, 2015. Rising temperatures reduce global wheat production. Nature Climate Change 5, 143–147.

Atanasova, D., N. Tsenov, I. Stoeva, I. Todorov, 2010. Performance of Bulgarian winter wheat varieties for main end-use quality parameters under different environments. Bulg. J. Agric. Sci., 16(1), 22-29.

Atanasova, D., N. Tsenov, I. Stoeva, V. Dochev, 2008. Genotype× environment interaction for some quality traits of Bulgarian winter wheat varieties. In Modern variety breeding for present and future needs. Proceedings of the 18th EUCARPIA general congress, Valencia, Spain, 9-12 September, 532-537.

Axinte, O., I., C. BadescuStroe, V.Neacsu, L. Bulgariu, D. Bulgariu, 2015. Evolution of trophic parameters from Amara Lake. J. Environ. Manag. 14, 559–565. https:// doi.org/10.30638/eemj.2015.060.

Barakova, T., G. Delchev, N. Valkova, 2018. Effect of Foliar Antibroadleaf Herbicides on Fat Content in Seeds of Various Cotton (*Gossypium Hirsutum* L.) Cultivars. Preprints 2018080017 (doi: 10.20944/preprints201808.0017.v1).

Blandino, M., F. Marinaccio, A. Reyneri, 2016. Effect of Late-season Nitrogen Fertilization on Grain Yield and on Flour Rheological Quality and Stability inCommon Wheat, under Different Production Situations. Italian Journal of Agronomy, vol. 2, 107-113. http://dx.doi.org/10.4081/ija.2016.745

Blandino, M.; F. Marinaccio, A. Reyneri, 2016. Effect of late-season nitrogen fertilization on grain yield and on flour rheological quality and stability in common wheat, under different production situations. Ital. J. Agron., 11, 107–113.

Bondarenko, M., M. Nazarenko, 2020. French breeding wheat varieties adaptabiliy for the Ukrainian North Steppe conditions. Agrology, 3(4), 193–198. doi: 10.32819/020022.

Bouazzama, B.; M. Boutfirass; A. Bahri; H. Lehamaoui, 2024. Effect of supplemental irrigation on yield and water productivity of durum wheat cultivated under drip irrigation in Tadla, Morocco. Moroccan Journal of Agricultural Sciences. DOI: 10.5281/ZENODO.10672577.

Chunsheng, Y., J. Li, Z. Zhang, Y. Liu, Z. Wang, Z. Sun, Y. Zhang, 2023. Improving wheat yield, quality and resource utilization efficiency through nitrogen management based on micro-sprinkler irrigation. Agricultural Water Management 108277

Delchev, G., S. Angelova, 2019. Productivity and Yield Stability at Late Treatment of Durum Wheat (Triticum durum Desf.) with Antibroadleaved Herbicides. III. Influence at Treatment during 3-rd Stem Node Stage. Agricultural Science and Technology, 11(1), 36-40. DOI: 10.15547/ast.2019.01.006.

Dimitrov, E., Z. Uhr, R. Dragov, R. Chipilsky, T. Angelova, 2023). Study of the elements of the productivity of old common winter wheat varieties under changing environmental conditions. Scientific Papers. Series A. Agronomy, 66(1), 299-306.

Eberhart, S., W. Russell, 1966. Stability parameters for comparing varieties. Crop Sci., 6, 36–40.

Edwards, M., J. Dexter, M. Scanlon, S. Cenkowski, 1999. Relationship of creep-recovery and dynamic oscillatory measurements to durum wheat physical dough properties. Cereal Chem. 76, 638–645. [CrossRef]

Frenken, K., Gillet, V., 2012. Irrigation Water Requirement and Water Withdrawal by Country. Food and Agriculture Organization, United Nations.

https://www.fao.org/worldfoodsituation/csdb/en

Kahiluoto, H. (2020). Food systems for resilient futures. Food Security, 12(4), 853-857.

Kahiluoto, H., J. Kaseva, J. Balek, J. Olesen, M. Ruiz-Ramos, A. Gobin, K. Kersebaum, J. Tak´aˇc, F. Ruget, R. Ferrise, P. Bezak, G. Capellades, 2019. Decline in climate resilience of European wheat. Proc. Natl. Acad. Sci. U. S. A. 116 (1), 123–128.

Kang, M. (1993). Simultaneous selection for yield and stability in crop performance trials: Consequences for growers. Agronomy Journal, 85, 754-757.

Kharrou M., R. Salah, C. Ahmed, D. Benoit, S. Vincent, L. Michel, O. Lahcen, J. Lionel, 2011. Water use efficiency and yield of winter wheat under different irrigation regimes in a semi-arid region. Agricultural Sciences in China, 2, 273-282.

Krystkowiak, K., M. Langner, T. Adamski, 2016. Interactions between Glu-1 and Glu-3 loci and associations of selected molecular markers with quality traits in winter wheat (*Triticum aestivum* L.) DH lines. Journal of Applied Genetics. Doi: 58. 10.1007/s13353- 016-0362-5.

Kuneva, V., E. Valchinova, M. Sabeva, 2014. Grouping of Local and Introduced Rye Samples and Their Output Lines on the Basis of Cluster Analysis. Agricultural Sciences, 10(24), 5-10.

Leghari, J., W. Han, A. Soomro, M. Shoukat, M. Zain, Y. Wei, Q. Xu, M. Buriro, T. Bhutto, R. Soothar, Y. Laghari, A. Laghari, 2024. Navigating water and nitrogen practices for sustainable wheat production by model-based optimization management systems: A case study of China and Pakistan, Agricultural Water Management, Volume 300, 108917, ISSN 0378-3774, https://doi.org/10.1016/j.agwat.2024.108917.

Li, H., J. Lin, L. Xue, Y. Ding, S. Wang, L.Yang, 2018. Fate of basal N under split fertilization in rice with 15N isotope tracer. Pedosphere 28, 135–143. https://doi.org/10.1016/s1002-0160(17)60407-7.

Lyu, K., Y. Liu, N. Li, L. Ku, Y. Hou, X. Wen, 2022. Foliar applications of various nitrogen (N) forms to winter wheat affect grain protein accumulation and quality via N metabolism and remobilization. Crop J. 10, 1165–1177. https://doi.org/10.1016/j.cj.2021.10.009.

Minoli, S., J. Jägermeyr, S. Asseng, A. Urfels, C. Müller, 2022. Global crop yields can be lifted by timely adaptation of growing periods to climate change. Nat Commun 13, 7079. https://doi.org/10.1038/s41467-022-34411-5

Morris, C., 2016. Evaluation of wheat-grain quality attributes. "Encyclopedia of Food Grains", pp. 251–256. doi:10.1016/b978-0-12-394437-5.00247-3.

Oliveira, A., G. Slafer, A. Fritz, R. Lollato, , 2020. Physiological basis of genotypic response to management in dryland wheat. Front. Plant Sci. 10, 1644.

Parveen, L., I. Khalil, S. Khalil, 2010. Stability parameters for tillers, grain weight and yield of wheat cultivars in north-west of Pakistan. Pakistan Journal of Botany, 42(3), 1613-1617.

Piepho, P., 2019. Recent claim of declining climate resilience in European wheat is not supported by the statistics used. Proc. Natl. Acad. Sci. 116 (22), 10625–10626.

Reckling, M., H. Ahrends, T. Chen, W. Eugster, S. Hadasch, S. Knapp, F. Döring, 2021. Methods of yield stability analysis in long-term field experiments. A review. Agronomy for Sustainable Development, 41, 1-28.

Rozbicki, J., A.Ooglińska, D. Gozdowski, 2014. Influence of the cultivar, environment and management on the grain yield and bread-making quality in winter wheat. Journal of Cereal Science, 61. Doi: 10.1016/j.jcs.2014.11.001.

Sevov A., V. Delibaltova, 2013. Effect of biostimulant fertigrain on bread wheat (*Triticum aestivum*) productivity elements and grain yield. Scientific papers series A. Agronomy, vol. LVI, 353-356, Bucharest, Romania.

Shi, L., D. Li, Q. Jing, J. Cai, D. Jiang, W. Cao, T. Dai, 2012. Effects of nitrogen applications on soil nitrogen balance and nitrogen utilization of winter wheat in a rice–wheat rotation. Field Crops Res. 127, 241–247. https://doi.org/10.1016/j.fcr.2011.11.025.

Shukla, G., 1972. Some statistical aspects of partitioning gen¬otype-environmental components of variability. Heredity, 29, 237–245.

Stoyanova, A., M. Georgiev, S. Atanasova, F. Emurlova, R. Mineva, 2020. Study of productivity and stability of yield of common wheat varieties, Journal of Mountain Agriculture on the Balkans, 2020, 23 (5), 75-88.

Tedone, L., S. Ali, L. Verdini, G. De Mastro, 2018. Nitrogen management strategy for optimizing agronomic and environmental performance of rainfed durum wheat under Mediterranean climate. J. Clean. Prod. 172, 2058–2074. https://doi.org/ 10.1016/j.jclepro.2017.11.215.

Trevisan, S.; A. Khorshidi, M. Scanlon, 2022. Relationship between nitrogen functionality and wheat flour dough rheology: Extensional and shear approaches. Food Res. Int., 162 Pt B, 112049.

Tsenov, N., D. Atanasova, 2015. Influence of environments on the amount and stability of grain yield in the modern winter wheat cultivars. II. Evaluation of each variety. Bulg. J. Agric. Sci., 21, 1128–1139.

Tsenov, N., K. Kostov, T. Gubatov, V. Peeva, 2004. Study on the genotype x environment interaction in winter wheat varieties. I. Grain quality. Field Crops Studies, 1(1), 20-29 (Bg).

Uhr, Z., E. Dimitrov, G. Delchev, 2021. Characteristics of perspective lines common winter wheat.1. Yield and stability. Rastenievadni nauki, 58(4), 3-10.

Uhr, Z., E. Dimitrov, R. Dragov, R. Chipilsky, T. Angelova, 2023. Comparative testing of old winter wheat varieties under changing climatic conditions. Scientific Papers. Series A. Agronomy, 66(1), 599-607.

Van Frank, G., P. Rivi`ere, S. Pin, R. Baltassat, J. Berthellot, F. Caizergues, C. Dalmasso, 2020). Genetic diversity and stability of performance of wheat population varieties developed by participatory breeding. Sustainability 12, 384.

Wan, X., W. Wu, F. Shah, 2021. Nitrogen fertilizer management for mitigating ammonia emission and increasing nitrogen use efficiencies by 15N stable isotopes in winter wheat. Sci. Total Environ. 790, 147587. https://doi.org/10.1016/j. scitotenv.2021.147587.

Yan, W., L. Hunt, 2001. Interpretation of genotype× environment interaction for winter wheat yield in Ontario. Crop Science, 41(1), 19-25.

Yao, C., J. Li, Z. Zhang, Y. Liu, Z. Wang, Z. Sun, Y. Zhang, 2023. Improving wheat yield, quality and resource utilization efficiency through nitrogen management based on micro-sprinkler irrigation. Agricultural Water Management, 282, 108277.

Zain, M., Z. Si, H. Ma, M. Cheng, A. Khan, F. Mehmood, A. Duan, C. Sun, 2023. Developing a tactical irrigation and nitrogen fertilizer management strategy for winter wheat through drip irrigation. Front. Plant Sci. 14:1231294. doi: 10.3389/fpls.2023.1231294.

Zhang, Z., Z. Yu, Y. Zhang, Y. Shi, 2021c. Split nitrogen fertilizer application improved grain yield in winter wheat (*Triticum aestivum* L.) via modulating antioxidant capacity and 13C photosynthate mobilization under water-saving irrigation conditions. Ecol. Process 10, 290–302. https://doi.org/10.1186/s13717- 021-00290-9.

Zubair, M., L. Akhtar, R. Minhas, M. Bukhari, S. Hussain, I. Ali, R. Ullah, 2021. Assessment of Wheat Genotypes for Quality Attributes Grown under Irrigated and Rainfed Conditions. Egyptian Journal of Agronomy, 43(1), 97-104.

Пахотина, И., Ю. Кашуба, Е. Игнатьева, В. Трипутин, 2020. Оценка на колекции озимой мягкой пшеница с качество на зърна. Вестник Алтайского государственного аграрного университета, 7 (189), 10-16.

Смирнова, В., 2018. Формирование технологических качеств зерна озимой пшеницы в Белгородской области / В. В. Смирнова, Н. А. Сидельникова, И. В. Кулишова. Инновации в АПК: проблемы и перспективы (1), 151-157.

Фоменко, М., 2018. Итоги селекции озимой мягкой пшеницы на качество зерна в условиях усиления флуктуации климата / М. А., Фоменко, А. И. Грабовец, Т. А. Олейникова, О. В. Мельникова. Известия Оренбургского (3), 59-63.

OPTIMIZING GERMINATION OF *NİGELLA SATİVA* L. WITH GIBBERELLIC ACID AND SEED PRIMING TECHNIQUES

Research Assistant Dr. Hatice Kübra GÖREN (ORCID: 0000-0001-7654-1450)

Email: hkubra.goren@adu.edu.tr

Aydın Adnan Menderes University, Faculty of Agriculture, Department of Field Crops

Aydın/ Türkiye

Research Assistant Dr. Uğur TAN (ORCID: 0000-0002-9592-2790)Email: ugur.tan@adu.edu.tr

Aydın Adnan Menderes University, Faculty of Agriculture, Department of Field Crops Aydın/Türkiye

Prof. Dr. Öner CANAVAR (ORCID: 0000-0003-4168-953X)

Email: ocanavar@adu.edu.tr

Aydın Adnan Menderes University, Faculty of Agriculture, Department of Field Crops AYDIN/TÜRKİYE

ABSTRACT

The use of *Nigella sativa* L., commonly known as black cumin, in traditional and medicinal practices is well-documented, due to the wide range of its biological activities. In light of its significance, this study investigates the efficacy of various seed priming treatments, specifically Gibberellic Acid (GA3) and different priming durations, under laboratory conditions to enhance the germination stages of *Nigella sativa* L. The objective of these priming treatments is to improve germination rates, the germination index, and average germination time, with the potential to increase agricultural productivity.
The findings of this study demonstrate that priming durations and gibberellic acid doses have a significant impact on germination parameters under controlled conditions. These results provide valuable insights into the potential of these treatments to optimise crop growth. It can be concluded that appropriate seed priming can effectively enhance the early growth stages of *Nigella sativa* L., contributing to better crop establishment and yield.

Keywords: Nigella sativa L., Seed priming, Gibberellic Acid (GA_3), Germination rates, Germination index

INTRODUCTION

Medicinal plants, especially those with a broad spectrum of biological activities, have attracted considerable attention in both the scientific and medical fields. *Nigella sativa* L., commonly known as black cumin or black seed and belonging to the Ranunculaceae family, is notable for its widespread use in culinary and medicinal contexts (Mohamed et al., 2014; Kiralan et al., 2016). Its medicinal use has attracted worldwide interest, largely due to its efficacy in the treatment of numerous conditions. Historical accounts, including those attributed to the Prophet Muhammad, have enhanced the reputation of black seed as a remedy for various ailments, except death itself (Islam et al., 2019).

Traditionally, *Nigella sativa* seeds have been used to treat conditions such as fatigue, persistent headaches and melancholy. Roasted seeds mixed with honey or butter have been suggested to relieve colic and coughs, while the seeds are known for their lactogenic and antiseptic properties, particularly in the treatment of eye infections. The emergence of COVID-19 has further sparked interest in potential antiviral treatments, with studies investigating Nigella sativa extracts for their ability to inhibit coronavirus replication and affect TRP gene expression during infection (Ulasli et al., 2014). The results suggest that *Nigella sativa* has a significant effect on viral load and gene expression associated with CoV infection, enhancing its therapeutic potential (Yimam et al., 2015).

The genus Nigella includes around 20 species, 14 of which are found in Türkiye (Nemtinov et al., 2022). It is cultivated globally, from Southern Europe to the Middle East and South Asia, and is particularly widespread in regions like Thrace, Northern Anatolia, and the Mediterranean in Türkiye, especially in provinces like Burdur, Afyon, Isparta, and Gaziantep (Inan et al., 2022).

Seed priming, a cost-effective method to promote early crop growth and uniform emergence, is a common agricultural practice. This involves treating seeds with osmotic solutions or plant growth promoters such as salicylic acid and gibberellic acid, or inorganic salts like KNO_3 and $CaCl_2$ (Farooq et al., 2008; Rehman et al., 2011). Priming typically accelerates germination, improves stand establishment, and enhances crop yield and quality. For example, KNO_3 priming not only speeds up germination and strengthens seedlings but also boosts yield and protein content when combined with other priming methods (Rehman, 2014).

A substantial body of research has demonstrated that a variety of priming techniques can positively influence seed germination and seedling growth (Paparella et al., 2015; Sharma et al., 2014; Khalaki et al., 2020). The primary objective of this study is to determine the optimal dose and duration of gibberellic acid (GA3) application for seed priming in Nigella sativa, with the aim of enhancing seed germination. While the study also aims to explore whether these optimal conditions observed in controlled laboratory settings can be effectively applied in field conditions, the central focus remains on identifying the most effective GA3 treatment parameters to improve seed germination.

MATERIAL AND METHOD

This study examines the impact of , Gibberellic Acid (GA_3), priming treatments on Nigella sativa L. seeds under controlled laboratory conditions. The seeds were subjected to germination tests, placed on four layers of moistened filter paper, and incubated in darkness at a constant temperature of 22°C. Germination was assessed by the emergence of a 1 mm radicle and monitored over a 10-day period. No signs of microbial contamination were observed during the experiment (Kamal et al., 2010).
For priming, there are four dose; Control dose, 100 ppm GA, 200 ppm GA and 300 ppm GA; three priming duration; 12 hour Priming, 24 hour Priming and 36 hour Priming applications. The experimental design was arranged in a completely randomized design with 4 replications of 25 seeds in the germination stage The seeds were placed in 10 × 10 cm plastic boxes lined with blotting paper.

Germination Rate (%): It is calculated as Scott et al., 1984.

$$GR = \frac{Number\ of\ germinated\ seeds}{Total\ number\ of\ seeds} * 100$$

Germination Index: The germination index is calculated by dividing the number of germinated seeds by the number of days to the first count, and adding that to the number of germinated seeds divided by the number of days to the final count (Benech Arnold et al., 1991).
Mean germination time (Day): It is calculated as Ellis &Roberts, 1981.

$$MGT = \frac{\Sigma(Dn)}{\Sigma n}$$

n : number of seeds germinated on day D,
D: number of days counted from the beginning of the germination the lightest seeds,
The obtained data were analyzed separately and differences were determined using the analysis of variance (one-way ANOVA). Variance analyses were performed using JMP Pro v.16 software. Statistically significant factors were grouped using the LSD (Least Significant Difference) test. Factors that were not significant in the LSD test were compared based on mean tables.

RESULTS AND DISCUSSION

The ANOVA analysis results highlight the significant effects of GA_3 (gibberellic acid) dosage and application time on the germination performance of Nigella sativa seeds. The results show that both factors have statistically significant impacts on parameters such as germination rate, germination index, and mean germination time. The GA_3 dosage has a highly significant effect on the germination rate at the 1% level ($p < 0.01$). Different GA_3 concentrations lead to noticeable differences in the seeds' germination success. Additionally, application time also significantly affects germination rate at the 1% level ($p < 0.01$), indicating that the duration of exposure to GA_3 plays an important role. However, the interaction between dosage and time was not statistically significant ($p > 0.05$), suggesting that the combined application of these two factors does not create a synergistic effect. GA_3 dosage significantly affects the germination index at the 5% level ($p < 0.05$), meaning that varying doses impact the rate of germination, though not as dramatically as for germination rate. On the other hand, application time did not significantly influence the germination index ($p > 0.05$), indicating that time does not largely affect germination speed. GA_3 dosage significantly affects the mean germination time at the 1% level ($p < 0.01$), showing that optimal dosages can shorten the time it takes for seeds to germinate. Similarly, application time also has a significant effect on the mean germination time at the 1% level ($p < 0.01$), indicating that longer exposure to GA3 can alter the germination process. However, the interaction between dosage and time did not significantly affect this parameter ($p > 0.05$) (Table 1).

These findings suggest that both GA_3 dosage and application time should be optimized independently to improve the germination performance of Nigella sativa seeds. The lack of a significant interaction between the two factors means that their combined application does not offer additional advantages, so it is important to focus on each factor separately for better results.

Table 1. Significance levels in variance analysis for the GA_3 priming dose applications and priming durations in *Nigella sativa* L. Plant.

Source of Variation	**Df**	**Mean of Square**		
		Germination Rate	**Germination Index**	**Mean germination time (Day)**
Rep.	**2**	21.33	0.127	0.048
Dose	**3**	564.74**	1.369*	0.173**
Time	**2**	112**	0.098	0.497**
Dose*Time	**6**	40.29	0.151	0.131
Error	**22**	21.33	0.9	0.053
Total	**35**			

The results presented in Table 2 show the effects of different GA3 doses and priming times on the germination parameters of mean value *Nigella sativa* seeds.

The 300 ppm GA_3 dose consistently yielded the highest germination rates across all priming times, with an average of 92.00%, significantly outperforming the control (72.88%) and lower GA_3 doses. The dose of 300 ppm GA_3 resulted in significantly higher germination compared to the other treatments ($p < 0.01$). When considering priming duration, 36-hour priming resulted in the highest average germination rate of 85.32%, followed by 12-hour (81.33%) and 24-hour (79.33%). The effect of the priming time was significant, indicating longer priming times positively influence germination rates. A similar trend is observed with the germination index. The highest index (4.05) was found in seeds primed with 300 ppm GA_3, indicating faster and more successful germination compared to the control (3.14) and other GA_3 concentrations. The effect of the dose on the germination index was statistically significant ($p < 0.05$). Among the priming durations, the 12-hour priming time slightly outperformed others with an average germination index of 3.57, though the differences between priming times were not as pronounced. Regarding the average germination day, seeds primed with 300 ppm GA_3 also exhibited the fastest germination, averaging 6.08 days. This was significantly faster compared to seeds primed with 200 ppm GA_3 (6.40 days) and the control (6.18 days). The effect of GA_3 dose on germination time was significant at $p < 0.01$. The longest germination time was recorded at the 36-hour priming duration (6.45 days), suggesting that longer priming may slow down the germination process, while shorter priming durations (12 and 24 hours) resulted in quicker germination.

Table 2. Effect of GA_3 priming dose applications and priming durations in *Nigella sativa* L. plant germination parameters.

GA Dose and Priming Time	**Germination Rate**				**Germination Index**				**Avarage Germination Day**			
	12 hour Priming	**24 hour Priming**	**36 hour Priming**	**Avg.**	**12 hour Priming**	**24 hour Priming**	**36 hour Priming**	**Avg.**	**12 hour Priming**	**24 hour Priming**	**36 hour Priming**	**Avg.**
Control dose	73.33	70.66	74.66	**72.88 C**	3.19	3.12	3.11	**3.14 C**	6.07	6.09	6.39	**6.18 AB**
100 ppm GA	81.33	72	86.66	**80.00 B**	3.61	2.94	3.5	**3.35 BC**	6.01	6.48	6.41	**6.30 AB**
200 ppm GA	78.66	84	86.66	**83.11 B**	3.33	3.64	3.35	**3.44 B**	6.24	6.09	6.87	**6.40 A**
300 ppm GA	92	90.66	93.33	**92.00 A**	4.17	3.89	4.08	**4.05 A**	5.89	6.2	6.15	**6.08 B**
Avg.	**81.33 B**	**79.33 b**	**85.32 A**		**3.57**	**3.39**	**3.51**		**6.05 B**	**6.21 B**	**6.45 A**	

CONCLUSION

The results of the analysis of GA_3 priming doses and durations on *Nigella sativa* seeds provide several key insights for optimising germination performance. The highest germination rates, germination index values and shortest average germination times were achieved with a GA_3 concentration of 300 ppm. This dose significantly improved germination performance compared to lower doses and the control, demonstrating its effectiveness in promoting faster and more successful seed germination. Of the different priming durations, 36 hours priming resulted in the highest average germination rate, but also resulted in a slightly longer average germination time. This suggests that while longer priming durations may improve the overall germination rate, they may also increase the time taken for seeds to germinate. Conversely, shorter priming durations (12 and 24 hours) tended to speed up the germination process, but with less pronounced improvements in germination index compared to the 36 hour priming.The results suggest that the GA_3 dose of 300 ppm combined with a 12- or 24-hour priming period may be the most balanced approach, providing high germination rates and relatively short germination times. The effect of the interaction between dose and priming time was not as significant as the individual effects of each factor.In conclusion, a GA_3 dose of 300 ppm is highly recommended to enhance the germination of *Nigella sativa* seeds, with 12 h or 24 h priming preferred for faster germination. Longer soaking times may be beneficial for overall germination rates but may slightly delay the germination process. Balancing these factors according to the specific needs of the seed and the desired results can lead to optimum germination performance.

REFERENCES

Farooq, M., Aziz, T., Hussain, M. & Khan, M. (2008). Glycinebetaine improves chilling tolerance in hybrid maize. Journal of Agronomy and Crop Science, 194(2), 152-160. https://doi.org/10.1111/j.1439-037x.2008.00295.x

Inan, M., Kırpık, M. & Büyük, G. (2023). The Effect of Different Locations on Black Cumin (Nigella sativa L.) Yield and Some Agricultural Characteristics in Adıyaman Province Conditions. Ziraat Mühendisliği, https://doi.org/10.33724/zm.1132314 .

Islam, M. T., Khan, R. & Mishra, S. K. (2019). An updated literature-based review: Phytochemistry, pharmacology and therapeutic promises of Nigella sativa L. Oriental Pharmacy and Experimental Medicine, 19(2), https://doi.org/10.1007/s13596-019-00363-3

Khalaki, M. A., Moameri, M., Behnam A. L. & Astatkie, T. (2020). Influence of nano-priming on seed germination and plant growth of forage and medicinal plants. Plant Growth Regulation, 93(1), 13-28. https://doi.org/10.1007/s10725 020-00670-9

Kiralan, M., Ulas, M., Özaydın, A. G., Ozdemir, N. & Ramadan, M. F. (2016). Changes in hexanal, thymoquinone and tocopherols levels in blends from sunflower and black cumin oils as affected by storage at room temperature. Rivista Italiana Delle Sostanze Grasse, 93(4), 229-236.

Mohamed F.R. Hassanien, M. F. R., Mahgoub, S. A. & El-Zahar, K. M. (2014). Soft cheese supplemented with black cumin oil: Impact on food borne pathogens and quality during storage. Saudi Journal of Biological Sciences, 21(3), 280-288. https://doi.org/10.1016/j.sjbs.2013.10.005

Nemtinov, V., Kostanchuk, Y., Motyleva, S., Pekhova, O., Timasheva, L., Pashtetskiy, V., … & Katskaya, A. (2022). Morphometric and biochemical assessment of nigella l. genotypes of european-asian origin. Journal of Breeding and Genetics, 54(3), https://doi.org/10.54910/sabrao2022.54.3.18

Paparella, S., de Sousa Araújo, S., Rossi, G. & Balestrazzi, A. (2015). Seed priming: State of the art and new perspectives. Plant Cell Reports, 34(8), https://doi.org/10.1007/s00299-015-1784-y

Rehman, H. Nawaz, Q., Basra, S. M. A. & Hassan, F. (2014). Seed Priming Influence on Early Crop Growth, Phenological Development and Yield Performance of Linola (Linum usitatissimum L.). Journal of Integrative Agriculture, 13(5), 990-996. https://doi.org/10.1016/s2095-3119(13)60521-3

Sharma, A., Rathore, S. V. S., Srinivasan, K. & Tyagi, R. (2014). Comparison of various seed priming methods for seed germination, seedling vigour and fruit yield in okra (Abelmoschus esculentus L. Moench). Scientia Horticulturae, 165(165), 75-81. https://doi.org/10.1016/j.scienta.2013.10.044

Ulasli, M., Gurses, S. A., Bayraktar, R. & Arslan, A. (2014). The effects of Nigella sativa (Ns), Anthemis hyalina (Ah) and Citrus sinensis (Cs) extracts on the replication of coronavirus and the expression of TRP genes family. Molecular Biology Reports, 41(3), 1703-1711. https://doi.org/10.1007/s11033 014-3019-7

Yimam, E., Nebiyu, A., Mohammed, A. & Getachew, M. (2015). Effect of Nitrogen and Phosphorus Fertilizers on Growth, Yield and Yield Components of Black Cumin (Nigella sativa L.) at Konta District, South West Ethiopia. Journal of Agronomy, 14: https://doi.org/10.3923/ja.2015.112.120

USE OF REAGENTS Na_2CO_3 AND NaOH IN THE PROCESS OF PURIFICATION OF RAW SALT WATER FOR THE PURPOSE OF PRECIPITATING Ca^{+2} AND Mg^{+2} IONS IN THE PRODUCTION OF QUALITY AND SAFE IODIZED SALT

Ema Obralić[1], Amela Semić[2], Arman Hodžić[3], Faris Muminović[4]

[1] *Faculty of Technology, University of Tuzla, Tuzla, Bosnia and Herzegovina*
[2] *Faculty of Agriculture and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina*
[3] *Veterinary Faculty Sarajevo, University of Sarajevo, Bosnia and Herzegovina*
[4] *AC FOOD d.o.o. , Velika Kladuša, Bosnia and Hezegovina*

Corresponding author e-mail: ema.obralic@solana.ba

With a well-planned and socially responsible business policy, Solana d.d. Tuzla, founded in 1885, has established itself as a key producer of salt on the Southeast European market. The process of producing salt, NaCl, is created by evaporating salt water in a four-stage vacuum evaporation battery. Raw salt water (SSV) is supplied to the saltwater pipelines circuit through a DN 250 pipe from the source of salt water in Tetima.
The aim of this research is to process all stages of the salt production process integrating all the requirements of the implemented standards. This paper will show the process approach and all the benefits of process management using GFSI principles.
When all the input parameters of the production process are satisfied, one of the most important process steps is the purification of raw salt water, which obtains a high-quality and safe essential product with all the required parameters. The purification process takes place in reactor 1DC/1,2. The process is based on the reduction of Ca^{+2} and Mg^{+2} ions within the permissible limits. The purification process itself takes place by mixing the salt water in the central pipe with the reagents Na_2CO_3 and NaOH, depending on the composition of the raw salt water. Purified salt water of satisfactory quality produced by process and laboratory monitoring is further transported to the vacuum evaporation plant and continues with the process stages of centrifugation, iodization, drying and screening of iodized salt.
The investigation confirmed that Solana d.d. Tuzla manages the process and the implemented requirements of the standards, ensuring traceability and the possibility of monitoring products in the complete chain of production and distribution. It bases its process management on the principles of sustainable technology using mother liquor as a by-product for further purpose and use.

Keywords: raw salt water, purified salt water, vacuum evaporation, mother liquor, iodization, traceability, sustainable technology, FSSC 22000

INTRODUCTION

Sodium chloride is one of the most common salts in nature. In crystalline form, it appears as rock salt in all geological formations. In Europe, large deposits of rock salt are found in Romania, Poland, Germany, France, England, Spain, and in Bosnia and Herzegovina in the territory of Tuzla. Exploitation of underground deposits is done by simply pumping out raw salt water through previously made wells, the composition of which can be variable. Raw salt water is transported by pipeline from the salt wells "Tetima" from the Tuzla Salt Mine and comes to the raw salt water receiving tank, then it is transported to the salt water purification plant by means of a pump through a meter using a pipeline. The process of purifying raw salt water is the removal of Ca^{2+} and Mg^{2+} ions within the permissible limits, and is carried out by the use of the reagents Na2CO3 and NaOH. The mixture of Na_2CO_3 and NaOH together with salt water passes through the already formed layer of sludge in the reactor, so that clear salt water (purified) from the top of the reactor goes into the salt water receiving tanks and goes directly to the four-stage vacuum evaporation station.
The process of purifying raw salt water and the continuation of all further processes is subject to monitoring and control based on GFSI principles. The salty slurry created by the process of vacuum evaporation is separated from the apparatus into the mixer, and from there it is transported to the concentrators by means of pumps and pipelines. Process control includes concentrator control and centrifuge operation control. Centrifuged salt is released from centrifuges on vibro - i.e. belt conveyors by which it is transported to the iodization drive. Salt is iodized at this plant. KJ or KJO_3 solution and an anti-caking agent are added to the salt. From the iodization plant, the salt from the belts falls into the dryer (vibrating dryer with a fluidizing layer), where the remaining moisture from the salt is extracted. Iodization of salt is carried out in order to prevent the occurrence of goiter in humans, domestic animals and livestock, which is caused by a lack of iodine. The process step of iodination is represented by CCP in the Production Plant itself. A by-product of salt production is mother liquor with a certain concentration of NaCl and Na_2SO_4. The mother liquor is discharged from the process when the concentration of Na2SO4 reaches the eutonic range, i.e. simultaneous crystallization of NaCl and Na_2SO_4.
The aim of the paper is to present the correlation between the GFSI principle and the basic step, i.e. purification of raw salt water through the traceability of the use of raw materials, reagents, additives and all the way to the finished product. In addition to the production of healthy and safe iodized salt, the goal is to point out the possibility of using mother liquor as a by-product of the salt production process.

MATERIAL AND METHODS

For experimental tests, raw salt water (SSV) was used, which was sampled at the discharge pipe of the snow pipeline in Solana, the composition of which is shown in Table 1.

Table 1. Raw salt water Solana d.d. Tuzla

Components	***NaCl (g/l)***	***Ca (g/l)***	***Mg (g/l)***	***HCO_3 (g/l)***	**SO_4^{2-} (g/l)**	***H_2S (mg/l)***
Reference values	***300-310***	***0,70-0,90***	***0,02-0,03***	***0,17-0,20***	***1,50-2,30***	***< 1***
Analysis results	*308,59*	*0,72*	*0,036*	*0,18*	1,39	0,44

If you look at the composition of raw salt water, Ca+2 and Mg+2 represent the carriers of hardness that must be reduced to the permitted limits because they affect the shift in the balance in the solution and reduce the coefficient of utilization of reactive NaCl, during the production of salt itself.
The amount of Na2CO3 and NaOH reagents is determined depending on the quality of the raw salt water, after the analysis as shown in Table 1. In the first stage of raw salt water purification, the reagents sodium hydroxide (NaOH) and sodium carbonate (Na2CO3) are used, and the process itself is called the soda-soda process.
The purification process takes place according to the following reaction mechanism:

$$Ca^{+2} + Na_2CO_3 \rightarrow CaCO_3 \downarrow + 2Na^+$$

$$Mg^{+2} + 2NaOH \rightarrow 2Na^+ + Mg(OH)_2 \downarrow$$

The reagents are prepared in a mixer where Na2CO3 and NaOH are dosed depending on the composition of the raw salt water and the flow rate, and they are transported by pump to the reactor where the salt water is purified. In the reactor with a contact layer, Ca2+ and Mg2+ are separated by mixing the salt water in the central pipe with the reactants Na2CO3 and NaOH, depending on the composition of the raw salt water. The mixture of Na2CO3 + NaOH together with salt water passes through the already formed layer of sludge in the reactor, so that from the top of the reactor the clear salt water (purified) goes to the receiving tanks of salt water and/or directly to the vacuum evaporation plant.
Iodization of salt is carried out in order to prevent the occurrence of goiter in humans, domestic animals and livestock, which is caused by a lack of iodine. Iodination is performed with a KJ or KJO3 solution in the range of 15 - 110 mg/kg of salt, depending on the customer's requirements. The iodination process is carried out according to the liquid-liquid principle, prepared solution of KJ or KJO3 on centrifuged salt. During salt production, the iodine content is monitored using iodometric titration according to the established sampling plan. The prescribed salt quality control methods according to the Codex standard for salt for human consumption are:

- Determination of NaCl content
- Determination of the content of insoluble substances in water/mineral acids
- Determination of sulfate content
- Determination of Ca^{2+} and Mg^{2+}
- Determination of H_2O content
- Determination of K^+ content

- Determination of heavy metal content - Cu (2.0 mg/kg)
- Determination of the content of heavy metals - As (0.5 mg/kg)
- Determination of the content of heavy metals - Hg (0.1 mg/kg)
- Determination of heavy metal content - Pb (2.0 mg/kg)
- Determination of heavy metal content - Cd (0.5 mg/kg)
- Determination of iodine content

One of the most important waste streams in salt production is mother liquor, and for these reasons, the treatment of mother liquor before its discharge and the possibility of using it in the process is of great importance for the sustainability of the salt production process. Table 2 shows the composition of raw salt water and mother liquor.

Table 2. Chemical composition and concentrations of raw salt water and mother liquor

Chemical composition	Raw salt water	Mother liquor
NaCl	308,59 gr/l	295,41 gr/l
Ca^{+2}	0,720 gr/l	63,01 gr/l
Mg^{+2}	0,03gr/l	0,198 gr/l
HCO_3^-	0,18 gr/l	1,14 gr/l
SO_4^{-2}	1,39 gr/l	1,06 gr/l

All tests were done so that the obtained results correspond to real systems.

RESULTS AND DISCUSSION

The process of purification of raw salt water after the dosing of reactants corresponds to the chemical composition shown in Table 3.

Table 3. Purified salt water

Components	***Ca* (mg/l)**	***Mg* (mg/l)**	***Na_2CO_3* (g/l)**	***NaOH* (g/l)**
Reference values	***30***	***5***	***0,30-0,40***	***0,10-0,15***
Analysis results	10	-	0,27	0,08

Since the process of purification of raw salt water is very important for the process of vacuum evaporation of salt water, great attention is paid to the control of purification, that is, analyzes of purified salt water. If we look at the reference values of purified salt water, and the values obtained after the purification procedure, we can conclude that the further process of vacuum evaporation will take place without inconsistencies in the process regarding the quality parameter of purified salt water.

Table 4. Chemical analysis of purified salt water produced by the applied purification method (mother liquor)[1]

Chemical composition	PSV after purification with ML
NaCl	306,66 gr/l
Na_2SO_4	19,88 gr/l
Na_2CO_3	0,90 gr/l
NaOH	0,017 gr/l
Ca^{+2}	0,0194 gr/l
Mg^{+2}	0,0021 gr/l
pH	9,63

Chemical analysis shows in Table 4 that all the parameters of the multicomponent system, such as purified salt water, are within the allowed limits, which confirmed the theoretical and experimental claims that part of the excess can be provided from the mother liquor.

After the production process is completed, the iodized table salt is transported to the silos from where it is mechanically packed. The quality of the packaging is visually controlled by the immediate machine operators (dispersion, var, declaration, date of packaging), and during the packaging, the weight and visual control of the packaged product is carried out by the packaging controller, while the physico-chemical characteristics are monitored in the test laboratories.

[1] 4. R. Babović, A. Brkić, E. Obralić, N. Balvanović, matična lužina kao odpadni tok u procesu proizvodnje soli, I. Naučno-stručni simpozij, Poljoprivredna proizvodnja i zaštita okoliša u funkciji razvoja ruralnih područja;

Table 5 shows the quality of salt that was produced and packaged on the day of the implemented traceability of salt production and packaging

DATA ON QUALITY			
No.	***Data***	***Quality conditions according to the rulebook***	***Analysis results***
1.	Content of pure NaCl on a dry basis	min. 97%	99,72
2.	Moisture content	max. 0.5%	0.03
3.	Foreign impurities visible to the naked eye	does not contain	No
4.	The smell	does not contain	No
5.	Color	White or slightly noticeable shade of another color	satisfies
6.	Mineral admixtures insoluble in hydrochloric acid	max. 0.05%	satisfies
7.	20% solution	Neutral to the litmus test	satisfies
8.	Contents of KJ or KJO3	Iodine mg/kg salt 15-23	19,04
9.	Granulation	min. 90% salt through a sieve with hole size 1.25 mm square	satisfies
10.	E - 536	max. 10 mg/kg	satisfies

According to the data presented in Table 5, the produced salt satisfies the quality and can be delivered to the final customer.

CONCLUSION

Based on the conducted analyses, following documentation and processes according to GFSI principles and in correlation with sustainable development standards, it is possible to conclude that Solana d.d. Tuzla adopted a business strategy that recognizes the necessity of adapting its business to the challenges posed by climate change and harmonizing with the global goals of sustainable development by 2030.

In the process of purifying raw salt water, the applied soda-soda process provides premium quality brine that allows the further flow of the process to take place without inconsistencies. The proportion of Ca^{+2} and Mg^{+2} is within the reference defined values, so that incrustations that affect the efficiency of the production process do not occur.

One of the critical control points is iodination. By meeting the iodine limits for exporting countries, Solana d.d. Tuzla represents a socially responsible company, because it markets a health-safe and safe product if we take into account that the state limits for all exporters range from 15 to 110 mgI/kg of salt. On the basis of the presented results in the part of the utilization of the mother liquor stream, it is clear that some basic requirements of the ESG strategy in the part of sustainable systems in the part of environmental protection are being respected.

This way of working, clear traceability in the process, utilization and use of own production process flows and returning to the system, it is possible that Solana d.d. Tuzla has competitiveness on the domestic and foreign markets and excellent business results, while at the same time respecting the social environment and the environmental component, striving to stop or minimize the harmful effects of its activities on the environment.

LITERATURE

1. S. Pribičević, Natrijum-hlorid, SODASO – Fabrika soli, 1975. Godina;
2. GS1 General Specifications, The foundational GS1 standard that defines how identification keys, data attributes and barcodes must be used in business applications. Release 16.0, Ratified, Jan 2016;
3. GS1 Logistics Label Guideline, Release 1.1, Ratified, Aug 2016;
4. R. Babović, A. Brkić, E. Obralić, N. Balvanović, Mother liquor as a waste stream in the process of salt production, I. Scientific and professional symposium, Agricultural production and environmental protection in the function of the development of rural areas;
5. V.M. Sedivy-Purification of salt for chemical and human consumption, Krebs Swiss, Zurich, Switzerland;
6. Arvind Kumar Indrajit, Process for the preparation of solar salt having high purity and whiteness, Patentdocs;
7. Chloride determination - volumetric method with silver nitrate with chromate indicator (Mohr's method); BAS ISO 9297:2002;
8. Internal documentation of Solana d.d. Tuzla;
9. Regulations on salt - exporting countries;
10. Standard for food grade salt, CODEX STAN 150-1985, Adopted in 1985; Revised in 1997; 2012; Amended in 1999; 2001; 2006
11. https://www.fssc.com/schemes/fssc-22000/

SYNTHESIS and CHARACTERIZATION of ACID-END GROUP POLY (ETHYLENE TEREPHTHALATE) COPOLYMER

Tuğba GÜNGÖR ERTUĞRAL[1], Cemil ALKAN[2]*

[1]Çanakkale Onsekiz Mart University, Çanakkale Faculty of Applied Sciences, Department of Food Technology, Çanakkale, Turkey
[2] Tokat Gaziosmanpaşa University, Faculty of Science and Letters, Department of Chemistry,Tokat, Turkey

**Corresponding author e-mail: tugbagungor@comu.edu.tr*

ABSTRACT

Food packaging material poly (ethylene terephthalate) (PET) is a polyester polymer that causes environmental pollution because it does not decompose for decades due to its inert and stable molecular structure. Synthesis of PET polymer with acid-end groups can help form a new material by allowing biodegradable polymers to be attached to this group. In this study, PET with acid-end group polymer was synthesized by esterification and polycondensation reactions by taking ethylene glycol (EG) and terephthalic acid (TPA) monomers in a 19:20 ratio and the obtained copolymer was characterized by Fourier Transform Infrared Spectroscopy (FTIR) and *Differential Scanning Calorimeters* (DSC). The FTIR spectrum displayed a prominent ester carbonyl (C=O) stretching peak at 1715 cm^{-1}. The melting temperature of the acid-end group PET copolymer was measured as 204.5 ºC and the melting enthalpy as 48.1 J/g. Based on the obtained results, PET polymer could be converted into a new generation polyester polymer with acid-end group.

Keywords: Food packaging material, poly (ethylene terephthalate), acid-end group, terephthalic acid, ethylene glycol

INTRODUCTION

Polyethylene terephthalate (PET) has become the ideal material for water and beverage bottles in the food industry and worldwide, with its unbreakable, lightweight and transparent properties, as well as its barrier properties against moisture and oxygen (Welle, 2011). PET production occurs in two different initial reactions: esterification of TPA and EG at temperatures between 240°C and 260°C and pressures between 300 and 500 kPa, and trans-esterification of dimethyl terephthalate (DMT) and EG at 150°C (Brydson, 1995). PET material is a thermally stable plastic material with good mechanical properties, chemical resistance, clarity, process ability and acceptability, as well as tensile and impact strength (Jankauskaite et al., 2008).

The world's plastic production, which was approximately 5 million tons/year in the 1950s, is now more than 280 million tons (Thompson et al., 2009; PlasticsEurope, 2011). Plastics and plastic waste, which are being monitored seriously today, have reached a point where they endanger marine biodiversity and the food chain, and therefore animal and human health (Thompson, 2015). Naturally microorganisms and genetically modified microorganisms may play a role in the degradation process of PET (Benavides et al., 2022).

PET's properties such as high crystallinity (30%-50%), high molecular weight, strong C-C bonds and hydrophobic surface are the main reasons for preventing biodegradation (Amobonye et al., 2021). The aromatic component of PET is important in its biodegradability and increases stability, chemical inertness and longevity (Yoshida et al., 2016). In the presence of microbial enzymes such as PET hydrolase, ester bond hydrolysis can break down PET into the simple form (Boneta et al., 2021; Gao et al., 2022). Additionally, some commercial lipases and esterases such as Rhizopus (Rh.) delemarlipase can hydrolyze some types of polyesters (Tokiwa et al., 1977) . The acid-end group PET polymer can be coupled to biodegradable polymers such as poly (ethylene glycol)(Güngör Ertuğral, 2016). In this study, acid-end group PET copolymer was obtained from EG and TPA monomers and characterized by FTIR and DSC.

MATERIAL AND METHOD

Preparation of acid-end group poly (ethylene terephthalate) copolymer

The esterification and polycondensation reactions of EG/TPA at the ratio of 19:20 were carried out in the acid-end group PET copolymer synthesis apparatus system and under nitrogen atmosphere with antimony trioxide (Sb_2O_3) as catalyst, respectively. The reaction was carried out in a four-necked flask placed in an oil bath and heated on the magnetic stirrer. In the first stage of the reaction, Sb_2O_3 was dissolved in EG and transferred to a four-necked flask and then TPA was added. The reaction was started at 60°C and the temperature of the mixture, which became homogeneous at 60 rpm at 30-50 psi pressure for 30 min, was increased to 160°C in 2.5 hours and then slowly to 280°C. Finally, a vacuum was applied to the system for 30 min. (Güngör Ertuğral, 2016).

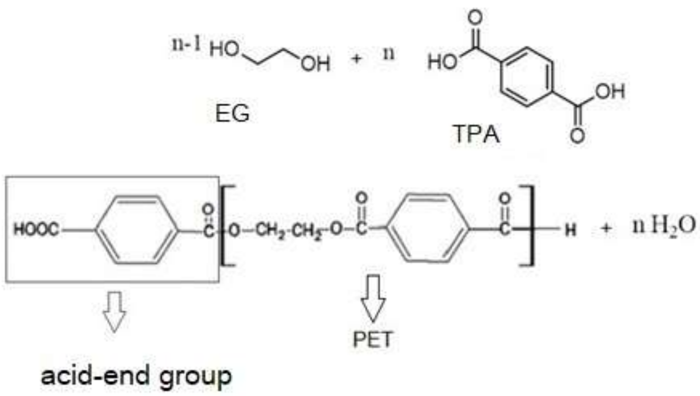


Figure1. Synthesis formula of acid-end group PET copolymer (Güngör Ertuğral, 2016).

DSC Analysis

Thermal properties of acid-end group PET were determined with a DSC Q2000 V24.11 Build 124 (TA Instruments, New Castle, Delaware, USA). 7 mg sample was prepared and heated and cooled at 5°C min-1 under nitrogen atmosphere between -28°C and 292°C (Chen et al., 2013; Sanchez-Silva et al., 2010).

FTIR Analysis

FTIR spectra were performed with a Perkin-Elmer FTIR Spectrum One B spectrometer (Waltham, Massachusetts, USA) and copolymer were recorded in transmission mode in a wavenumber range of 4000–600 cm^{-1} with a spectral resolution of 4 cm^{-1} (Chen et al., 2013; Khan et al., 2018).

RESULTS AND DISCUSSION

The obtained polymers were characterized by FTIR and DSC.

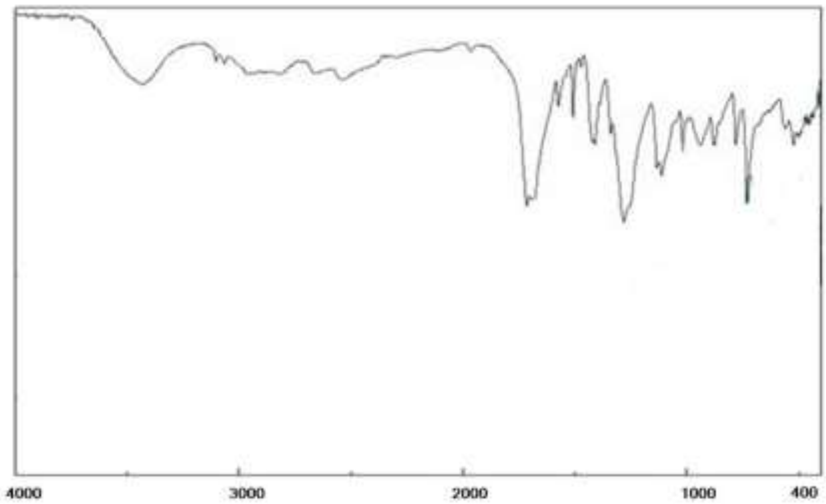


Figure1. FTIR spectrum of acid-end group PET

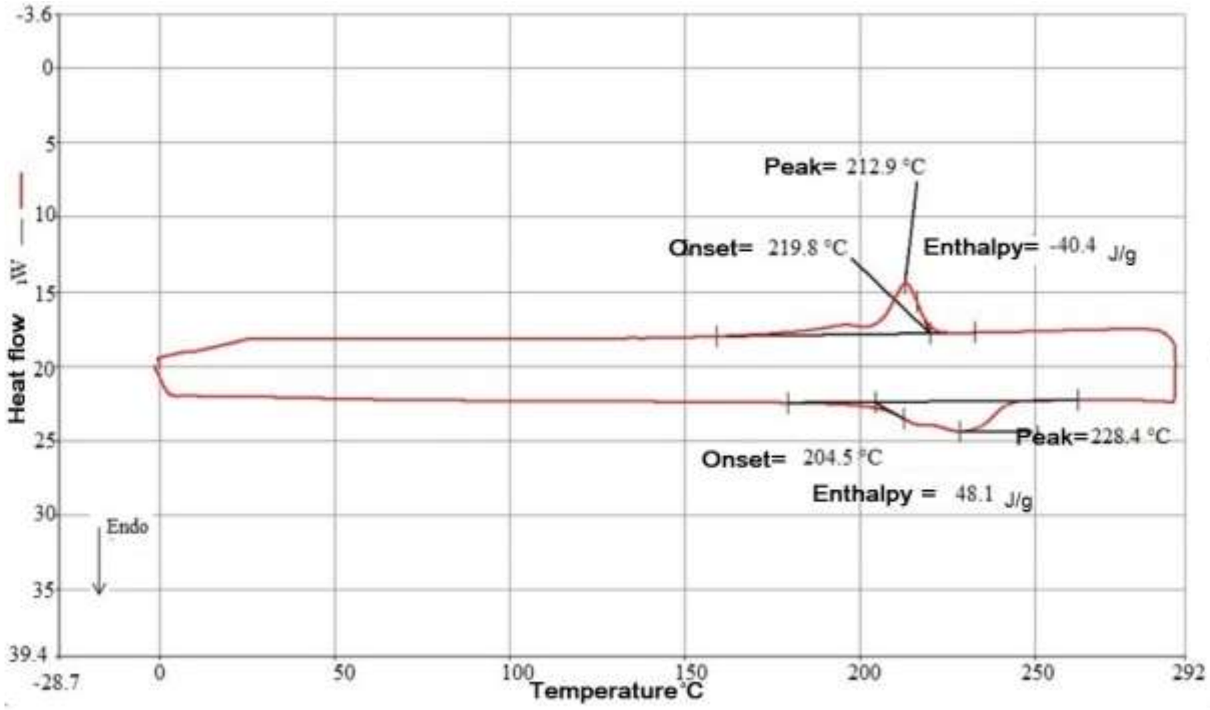


Figure 2. DSC curve of acid-end group PET

In the FTIR spectrum in Figure 1, a distinct ester carbonyl (C=O) stretching peak at 1715 cm^{-1} and a C-H stretching peak at 2870 cm^{-1} are clearly seen. According to the DSC curves in Figure 2, melting temperature of the acid-end group PET is 204.5°C and solidification temperature is 219.8°C, melting enthalpy is 48.1 J/g and solidification enthalpy is -40.4 J/g (Table 1).

Table1. Thermal properties of acid-end PET copolymers

Copolymer	Melting Temperature ºC	Melting Enthalpy(J/g)	Solidification Temperature ºC	Solidification Enthalpy(J/g)
Acid-end group PET	204.5	48.1	219.8	-40.4

CONCLUSIONS

In this study, acid-end group PET copolymer was successfully synthesized. The degradation of PET polyester polymer with its resistant molecular structure takes decades and causes serious environmental pollution. When biodegradable polymers are attached to the synthesized acid-terminated PET copolymer, new generation environmentally friendly materials can be obtained.

Acknowledgment: In this study, an excerpt was made from the doctoral thesis numbered 430843 of the Chemistry Department of the Tokat Gaziosmanpaşa University, Institute of Science.

REFERENCES

Amobonye, A., Bhagwat, P., Singh, S., & Pillai, S. 2021. Plastic biodegradation: Frontline microbes and their enzymes. Science of the Total Environment, 759, 143536.

Benavides Fernández, C. D., Guzmán Castillo, M. P., Quijano Pérez, S. A., & Carvajal Rodríguez, L. V. 2022. Microbial degradation of polyethylene terephthalate: a systematic review. SN Applied Sciences, *4*(10), 263.

Boneta, S., Arafet, K., & Moliner, V. 2021. QM/MM study of the enzymatic biodegradation mechanism of polyethylene terephthalate. *Journal of Chemical Information and Modeling*, *61*(6), 3041-3051.

Brydson, J. A. 1995. Thermoplastic elastomers: properties and applications. iSmithers Rapra Publishing, Vol.81.

Chen, C., Liu, K., Wang, H., Liu, W., & Zhang, H. 2013. Morphology and performances of electrospun polyethylene glycol/poly (dl-lactide) phase change ultrafine fibers for thermal energy storage. Solar energy materials and solar cells, 117, 372-381.

Gao, R., Pan, H., Kai, L., Han, K., & Lian, J. 2022. Microbial degradation and valorization of poly (ethylene terephthalate)(PET) monomers. *World Journal of Microbiology and Biotechnology*, *38*(5), 89.

Güngör Ertuğral, T. 2016. Preparation of polyester based and protective food packaging materials with thermal energy storage property through solid-solid phase changes. Doctoral thesis, Department of Chemistry of the Institute of Science of Tokat Gaziosmanpaşa University. https://tez.yok.gov.tr/UlusalTezMerkezi

Jankauskaite, V., Macijauskas, G., & Lygaitis, R. 2008. Polyethylene terephthalate waste recycling and application possibilities: a review. Mater Sci (Medžiagotyra), 14(2), 119-127.

Khan, S. A., Khan, S. B., Khan, L. U., Farooq, A., Akhtar, K., & Asiri, A. M. 2018. Fourier transform infrared spectroscopy: fundamentals and application in functional groups and nanomaterials characterization. Handbook of materials characterization, 317-344.

Plastics Europe Market Research Group. 2011. Plastics–the Facts 2011–An analysis of European plastics production, demand and recovery for 2010. *Plastic Europe Association of Plastic Manufacturers*.

Sanchez-Silva, L., Tsavalas, J., Sundberg, D., Sánchez, P., & Rodriguez, J. F. 2010. Synthesis and characterization of paraffin wax microcapsules with acrylic-based polymer shells. Industrial & engineering chemistry research, 49(23), 12204-12211.

Thompson, R. C. 2015. Microplastics in the marine environment: sources, consequences and solutions. Marine anthropogenic litter, 185-200.

Thompson, R. C., Moore, C. J., Vom Saal, F. S., & Swan, S. H. 2009. Plastics, the environment and human health: current consensus and future trends. Philosophical transactions of the royal society B: biological sciences, 364(1526), 2153-2166.

Tokiwa, Y., & Suzuki, T. 1977. Hydrolysis of polyesters by lipases. *Nature*, *270*(5632), 76-78.

Welle, F. 2011. Twenty years of PET bottle to bottle recycling—An overview. *Resources, Conservation and Recycling*, *55*(11), 865-875.

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., ... & Oda, K. 2016. A bacterium that degrades and assimilates poly (ethylene terephthalate). Science, 351(6278), 1196-1199.

UTILIZATION OF POMEGRANATE JUICE PRODUCTION WASTE IN DIETARY SUPPLEMENT TABLET PRODUCTION: EFFECTS OF BINDER RATIO AND WASTE PARTICLE SIZE ON TABLET QUALITY

Gökçe KAHRAMAN[1], Bilge TAŞKIN[2], Nazli SAVLAK[1]

[1] Department of Food Engineering, Faculty of Engineering, Manisa Celal Bayar University, 45140, Manisa, Turkey
[2] Technology Transfer Office, Rectorate, Çankırı Karatekin University, Uluyazı Campus, Merkez, 18100, Çankırı, Turkey
Corresponding author e-mail: nazli.yeyinli@cbu.edu.tr

ABSTRACT

Pomegranate (Punica granatum L.) is a perennial plant belonging to the Punica genus of the Lythraceae family, with a cultivation history dating back to 3000 BCE. It is native to Iran and is known to grow in the Middle East, the Caucasus, and northern India, encompassing tropical and subtropical climates, including the southern and southeastern regions of Turkey. The pomegranate is consumed fresh but can also be processed into products such as juice, juice concentrate, jam, wine, and liqueur, and used as a coloring and flavoring agent in various foods. After processing, approximately half of the fruit is discarded as waste, consisting of pulp, rind, and seeds. It is known that production wastes are rich in phenolic compounds and dietary fiber. This study determined some physical and chemical properties of dietary supplements pressed using a single-punch tablet press machine with pomegranate juice production waste powders of three different particle sizes (<500, <710, <1000 µm) and three different ratios of binder (20%, 30%, 40% Polyethylene Glycol (PEG) 4000). The bulk densities of the powder mixtures ranged from 0.67 to 0.72 g/cm³, the tapped densities ranged from 0.78 to 0.86 g/cm³, Carr Index values ranged from 10.71 to 17.24, Hausner ratios were between 1.12 and 1.21, angle of repose ranged from 32.23° to 35.74°, flow rates were between 0.90 and 1.97 mm/s, and water absorption varied from 70.99% to 122.29%. The hardness of the dietary supplements (429 - 4849 g force) increased with the PEG 4000 ratio, with the highest hardness observed at 40% PEG 4000 and particle size of <710 µm. The friability of the tablets ranged from 0.45% to 2.76%, with the lowest friability (0.45%) observed at 40% PEG 4000 and <710 µm particle size, as expected with the highest hardness. Notably, under conditions of reduced PEG 4000 ratio and particle size (<500 µm), the lowest hardness and highest friability were observed. Considering the significant quality indicators of high hardness and low friability, it was concluded that the highest quality tablets were obtained using 40% PEG 4000 with pomegranate juice production waste powder particle size of <710 µm.

Keywords: pomegranate, waste, dietary supplement, tablet quality

INTRODUCTION

Today, nearly all process wastes are either destroyed or repurposed as fertilizer or animal feed. Among these, the waste generated from pomegranate juice production, which has limited economic value, constitutes a significant portion. Approximately 50% of the waste produced from pomegranate processing consists of peel, pulp, and seeds (Figure 1-3), with the peel accounting for 33-40% of the fruit's total weight, although this proportion can vary depending on the fruit variety (Topkaya, 2012). Recently, pomegranates have garnered considerable attention from researchers due to their high content of phenolic compounds. Consequently, there has been a growing interest in utilizing pomegranate peel in food applications, particularly as a functional ingredient due to its potential as a natural antioxidant.

Figure 1. Pomegranate

Figure 2. Pomegranate peels

Figure 3. Pomegranate juice pulp

It has been reported that wastes generated from pomegranate juice production possess higher antioxidant activity, phenolic components, and bioaccessibility compared to pomegranate fruit itself, making them suitable for use as functional ingredients in food supplements or food products (Surek and Nilüfer-Erdil, 2016). Specifically, pomegranate peel is characterized by a composition of 70% moisture, 4% protein, 31% sugar, 28% fiber, and 5% ash on a dry weight basis (Uca, 2019). Chemical composition of pomegranate peel constitutes carbohydrates (80,50–94,90 g/100 g DM (dry matter)), crude fibers (11,22–34,00 g/100 g DM), crude protein (1.30–8.72 g/100 g DM, crude fat (0,50-9,40 g/100 g DM), ash (3,0–5,00 g/100 g DM). Pomegranate peels are notably rich in secondary metabolites, particularly phenolic compounds such as phenolic acids, flavonoids, and hydrolyzable tannins. These include a variety of bioactive compounds like condensed tannins, ellagitannins, gallotannins, ellagic acid, gallic acid, anthocyanins, kaempferol, luteolin, and naringin (Kaderides et al. 2021, Uca, 2019). The peel is packed with vital minerals like potassium (K), calcium (Ca), phosphorus (P), and sodium (Na), along with notable levels of iron (Fe), zinc (Zn), copper (Cu), and selenium (Se). It is also good source of vitamins such as B1 (thiamine), B2 (riboflavin), C (l-ascorbic acid), E (α-tocopherol), and A (retinol) (Rowayshed et al., 2013). The concentrations and quantities of these compounds vary with fruit ripening, environmental conditions, and the pomegranate variety.

Modern medical research provides evidence supporting the potential use of pomegranates in treating a range of conditions, including cancer, cardiovascular disease, diabetes, Alzheimer's disease, infertility, arthritis, and obesity, as well as for protection against ultraviolet (UV) radiation. The health benefits are believed to arise from the synergistic interactions of various bioactive compounds present in pomegranates and their derivatives (Jurenka, 2008).

Food supplements encompass a variety of components including vitamins, minerals, amino acids, and herbal ingredients, all of which are intended to support an individual's dietary intake. These products often contain elements such as antioxidants derived from fruits and vegetables, fiber from grains, and proteins from dairy sources. While food supplements are not classified as pharmaceuticals, their appropriate use can contribute positively to health (Kazaz, 2020) and these supplements are formulated in various oral forms, including tablets, capsules, and liquids (Atalay and Erge, 2018). Specifically, tablets are produced by compressing homogeneous powder mixtures of active ingredients and excipients using specialized machinery. Tablets are the one of the mostly used pharmaceutical dosage form in the drug industry due to their advantages, including cost-effective mass production from solid active ingredients, ease of use, precise dosing accuracy, and flexibility in terms of shape, color, and flavor. They also offer long shelf life, ease of packaging, transport, and storage, with the possibility of modifying active ingredient release through diverse formulations and production techniques (Kumar et al., 2002).

This study aims to evaluate the feasibility of using pomegranate peel as a functional ingredient for food supplements by investigating its chemical composition and physical properties in tablet formulations. It focuses on optimizing the effects of Polyethylene Glycol (PEG) 4000 ratios and peel powder particle sizes on tablet quality, including bulk density, flowability, tablet hardness and friability. The findings will provide insights into sustainable use of pomegranate peel and its potential in developing antioxidant-rich supplements. The next part of the study can be to analyze the nutritional value of the tablets, emphasizing dietary fiber, phenolic content, antioxidant capacity, and bioacceptability.

MATERIAL and METHOD

Materials

The pomegranate juice by-products (peels) used in the study were obtained from Semes Gıda San ve Tic. Ltd. Şti, Izmir, Turkey immediately after pomegranate juice production.
,

Pomegranate Peel Powder Production

The pomegranate juice production by-products were dried at 40°C in a tray dryer (Figure 4), ground into powder, and sieved (Figure 5) to obtain particle sizes determined by preliminary trials (<500, <710, and <1000 µm). The resulting pomegranate peel powder, with a moisture content of 7,05%, ash content of 2,98%, dietary fiber content of 14,1%, and protein content of 3,52%, was stored at +4°C for physical and chemical analyses.

Figure 4. Dried pomegranate peels

Figure 5. Pomegranate peel powder

Directly Compressed Tablet Production

Polyethylene glycol 4000 (PEG 4000) was utilized as the binder in the formulation process. Based on preliminary trials, it was determined that PEG 4000 should be used in particle sizes smaller than 1000 µm for all samples. Additionally, pomegranate peel particles were categorized into three distinct sizes: <500 µm, <720 µm, and <1000 µm. The optimal PEG 4000 concentration was established at 20%, 30%, and 40% through preliminary testing.

The tableting process for the mixtures (PEG 4000 and pomegranate peel powder) was performed manually using the direct compression method with a single-punch tablet press machine (Renas Machinery, Istanbul), located in the Food Engineering Laboratory at Manisa Celal Bayar University (Figure 6). A minimum of 50 tablets were produced from each sample. Each tablet was manufactured to have a weight of 1,25 ± 0,05 g. Following production, the tablets were stored in airtight glass jars for subsequent analysis.

Figure 6. Single punch tablet press

Tablet (Mixture) Powder Analyses

Color

The CIE L* (lightness), a* (red/green), and b* (yellow/blue) values of the pomegranate peel powders were determined using a Konica Minolta CR5 Chromameter (Japan) with a D65 light source and a 100-degree measurement angle. Color analysis was conducted by measuring at five different points over three separate repetitions, and the results were averaged (Ho et al., 2013).

Bulk Density and Tapped Density

The bulk density was determined by transferring a known mass of pomegranate peel (average 2 grams) into a 10 mL graduated cylinder from a specific height and measuring the volume it occupied. The bulk density was then calculated by dividing the mass of the pomegranate peel by the volume it occupied. For the tapped density, a known mass of pomegranate peel (average 2 gr) was placed into a 10 mL graduated cylinder. The powder was compacted using a glass rod until the volume it occupied remained constant at a fixed time and speed. The minimum volume occupied by the powder was then measured. The tapped density and volume were calculated considering the weight of the powder (Jinapong et al., 2008).

Carr's Compressibility Index (Carr Index) and Hausner Ratio (HR)

The Carr's compressibility index and Hausner ratio are rapid, simple, and cost-effective indirect methods used to determine the flow properties of powder granules. In this study, the Carr Index and Hausner Ratio were calculated to assess the flowability and cohesiveness of the powder samples (Jinapong et al., 2008). The compressibility of the powder is significant in tablet production as it influences tablet thickness (Ofori-Kwakye et al. 2010).

Carr Index was calculated according to the equation below. CI% between 5-12% corresponds to perfect powder flow, whereas 13-16% indicates good powder flow properties. A Carr Index bigger than 36% represents a very poor flow ability of a powder (Gandhi, 2012).

CI%= [(powder volume before compression - compressed powder volume)/powder volume before compression)] x100

Hausner ratio (HR) was calculated according to the equation below. A Hausner ratio <1.25 indicates that powders have good flow properties, while a Hausner ratio >1.25 indicates that powders have poor flow properties (Gandhi, 2012).

HR= tapped density/bulk density (Jinapong et al., 2008).

Flow Rate and Angle of Repose

Powders of known weight were poured from a certain height with the help of a funnel. Flow time was measured with a stopwatch (mm/s). The analysis was repeated 10 times. Angle of repose was determined by flowing the powder granules down a funnel fixed at a height of 5 cm from the ground (so that the tip of the funnel was 5 cm). Angle of repose indicates the cohesive property of a powder, and there is an inverse relationship between the angle of repose and flowability. The angle of repose (θ) was calculated on the equation below by measuring the height (h) and radius (r) of the dust accumulation (Aslani and Daliri, 2016).

$\tan \theta = (h / r)$ where θ is the angle of repose, h is the height, r is the radius.

Moisture and Ash Content

Moisture content was determined in accordance with the AACC 44-15.02 method and calculated as the weight difference after drying/total peel powder *100. Ash and dietary fiber contents were determined according to the mehods AACC 08-01.01 and 32-05-01 (AACC 2010) and calculated as % dry matter.

Tablet Analyses

Diameter, Thickness, and Weight

The average diameter, thickness, and weight of the tablets were analyzed according to the method described by Bushra et al. (2008). Twenty randomly selected tablets were weighed using a precision scale to determine the average tablet weight. The thickness and diameter of the 20 tablets were measured with an electronic caliper, and the average tablet thickness and diameter were calculated. The resulting values are reported as the mean values in millimeters (mm).

Color

The CIE L* (lightness), a* (red/green), and b* (yellow/blue) values of the tablets were determined using a Konica Minolta CR5 Chromameter (Japan) with a D65 light source and a 100-degree measurement angle. Color analysis was performed by measuring at five different points across three separate repetitions, and the results were averaged (Ho et al., 2013).

Tablet Hardness and Friability

The hardness of the tablets was assessed using a Texture Analysis Device (TA-XT Plus Texture Analyzer, Stable Micro Systems, Godalming, England) with a 30 kg load cell. Tablets were placed in a vertical position under a 25 mm cylindrical probe and compressed at a rate of 0,03 mm/s over a distance of 1 mm. The maximum force at the point of tablet fracture (g force) was used to represent the tablet hardness. The analysis was conducted on 20 tablets to determine the average tablet hardness, with the values reported as the mean values in g force.

Friability in tablets can develop due to friction and mechanical impact. Friability is directly related to tablet hardness; increased hardness can reduce durability and increase wear. The analysis was conducted as a service provided by the Pharmaceutical Sciences Research Laboratory (FABAL) at the Faculty of Pharmacy, Ege University, following the method of (Osei-Yeboah and Sun, 2015).

RESULTS AND DISCUSSION

Moisture and Ash Content of Tablet Powders

After the pomegranate peel was dried and crushed into powder, chemical composition analysis revealed that the moisture content was 7,05%, the protein content was 3,52%, the ash content was 2,98% and the dietary fiber content was 14,1%. Kushwaha et al. (2013) determined the ash, protein, and crude fiber content of the pomegranate peel to be 5,49%, 3,95%, and 12,61%, respectively, which is in line with our findings, considering the differences in climate and soil conditions.

Table 1 presents the moisture and ash content of powders with varying PEG 4000 ratios and pomegranate peel particle sizes. High moisture content can affect the flowability and stickiness of a powdered product. The obtained tablet powders, with moisture levels between 4,43% and 7,37%, were found to be sufficiently dry. Increasing the ratio of PEG 4000 resulted in a lower moisture and ash contents in the powders. On the other hand, the moisture content reached its maximum at the largest peel powder particle size (hereafter referred to as "particle size").

Table 1. Effect of PEG 4000 ratio and pomegranate peel powder particle size on moisture, ash contents and color values of the tablet powder

Sample #	PEG ratio (%)	Peel powder particle size (µm)	Moisture (%)	Ash (%)	*L**	*a**	*b**
1	20	<500	6,57	3,15	57,21	12,16	25,87
2	30	<500	5,95	2,53	57,77	11,80	25,05
3	40	<500	4,82	2,03	58,74	11,49	24,62
4	20	<710	6,04	2,74	58,53	11,34	24,16
5	30	<710	5,38	2,49	58,21	10,29	21,93
6	40	<710	4,43	2,00	58,65	10,91	22,48
7	20	<1000	7,37	3,00	54,86	11,38	23,48
8	30	<1000	6,46	2,52	54,80	11,75	23,30
9	40	<1000	5,68	2,49	56,38	10,81	22,04

Color Values of Tablet Powders

The L^* (lightness) values of the samples ranged between 54.80 and 58.74. The lowest lightness values were observed for the samples having the pomegranate peel powders with the largest particle size (<1000 µm). The PEG ratio and particle size had no significant effect on the a^* and b^* color values (Table 1.)

Physical Properties of Tablet Powders

Determining the flowability and adhesion behavior of powdered products is important for processes such as packaging, processing, storage, dosing, and mixing. High moisture content affects the flowability of the powdered product. Additionally, products with a high degree of adhesion tend to experience flowability issues. The bulk and tapped density results provide important insights into the compressibility of powder mixtures during tablet production and also offer valuable information regarding the strength of the tablets. Therefore, it is expected that the tablet mixtures will have an adequate level of density.

The bulk and tapped density of the powder mixtures reached their highest values in tablet powders containing the smallest peel particle sizes (Table 2). A similar effect was observed by Zhong et al. (2016) in pomegranate peel powders ground and sieved into different sizes, where the bulk density ranged from 0.653 to 0.751 g/mL. Low molecular weight PEGs undergo greater densification and form compacts of low porosity which results in increased hardness (Larhrib et al., 1997). However, in this study PEG ratio did not pose a significant effect on the density of the tablets.

Table 2. Effect of PEG 4000 ratio and pomegranate peel powder particle size on the physical properties of the tablet powder.

Sample #	**PEG ratio (%)**	**Peel powder particle size (µm)**	**Bulk density (g/cm^3)**	**Tapped density (g/cm^3)**	**Carr index (CI%)**	**Hausner ratio (HR)**	**Angle of repose (θ)**	**Flow rate (mm/s)**
1	20	<500	0,70	0,81	13,79	1,16	32,91	1,75
2	30	<500	0,68	0,82	16,67	1,20	33,78	0,90
3	40	<500	0,71	0,86	17,24	1,21	34,12	1,29
4	20	<710	0,71	0,85	15,79	1,19	32,23	1,18
5	30	<710	0,70	0,81	13,79	1,16	33,41	1,87
6	40	<710	0,72	0,81	10,71	1,12	35,03	1,85
7	20	<1000	0,69	0,81	13,79	1,16	35,74	1,97
8	30	<1000	0,67	0,78	13,33	1,15	34,92	1,87
9	40	<1000	0,68	0,78	13,33	1,15	34,36	1,71

The Carr Index (CI%) indicates the flowability of the powder, while the Hausner Ratio provides information on the powder's cohesiveness, which in turn affects its flowability. A high cohesiveness in a powder typically implies poor flowability (Koç et al., 2011). Jinapong et al. (2008) stated that powders with a Carr Index below 15 have very good flowability, while those with a Carr Index between 15 and 20 have good flowability. The results obtained in this study indicate that the flowability of all pomegranate peel powders is classified as either good or very good. Powders with a Hausner Ratio below 1,2 are categorized as having low cohesiveness, those between 1,2 and 1,4 have moderate cohesiveness, and those above 1.4 are classified as having high cohesiveness. The cohesiveness values obtained in this study, except for sample 3, are below 1,2, indicating that the powders have low cohesiveness. As the particle size of the peel powder increased from 500 to 1000 µm, both the Carr Index and Hausner Ratio values decreased, and flow rate increased, indicating an improvement in flowability (Table 2). Similarly, Metiner et al. (2023) reported that pomegranate peel powders dried under different microwave powers and drying temperatures exhibited very good flowability and low cohesiveness.

Gandhi (2012) defined powder flowability as follows: excellent for powders with an angle of repose below 20º, good for those between 20º and 30º, fair for those between 30º and 40º, and poor for those above 40º. In our study, all samples were found to be flowable enough based on their angle of repose (34,055 º, in average).

Post-compression Properties of the Tablets

Weight, Diameter, and Thickness of the Tablets

In the production (compression), the tablets were adjusted to a weight of 1.25 ± 0.05 g, and it was verified that their weights fell within this range (Table 3). The diameters of the tablets ranged from 13.32 to 13.47 mm, and their thicknesses varied from 8.66 to 8.90 mm. Overall, there appears to be no significant effect of PEG ratio or pomegranate peel particle size on the thickness of the tablets.

Hardness and Friability of the Tablets

Table 3 displays the hardness and friability of the direct compression tablets. Tablet hardness is a parameter that varies depending on the characteristics of the final powder and the compression force of the tablet press (Canbağı et al., 2016). It provides information about the tablet's durability and significantly affects the tablet's disintegration time and, consequently, the dissolution rate of the active ingredient. Tablet friability is the tendency of a tablet to lose component particles due to abrasion, friction, or mechanical shock. Ideally, ≤1% weight loss is acceptable for an existing compressed and uncoated tablet product (Osei-Yeboah and Sun, 2015). Friability also provides information about the tablets' durability under conditions such as handling, storage, and transport.

An increase in the PEG 4000 ratio resulted in greater tablet hardness and reduced friability. The results of the study indicate that the tablets with suitable friability values (that is ≤1%) are those with sample codes 5, 6, and 9. None of the samples with pomegranate peel particle sizes of 500 µm or less, and those with a PEG 4000 ratio of 20%, had an accepted friability value. Therefore, it was determined that a PEG 4000 ratio of 40% or higher was appropriate for pomegranate peel powder tablets, with particle sizes below 710 µm and 1000 µm, including medium and large particles.

Tablets with these characteristics not only exhibit physical durability but also have the potential to be dietary supplements due to their content of dietary fiber, total phenolic compounds, and high antioxidant capacity.

Table 3. Effect of PEG 4000 ratio and pomegranate peel powder particle size on the post-compression properties of the tablets.

Sample #	PEG ratio (%)	Peel powder particle size (µm)	Weight (g)	Diameter (mm)	Thickness (mm)	Hardness (g force)	Friability (%)
1	20	<500	1,22	13,47	8,89	429	2,76
2	30	<500	1,27	13,45	8,78	1490	1,47
3	40	<500	1,27	13,38	8,66	2941	1,15
4	20	<710	1,29	13,44	8,85	1386	1,45
5	30	<710	1,30	13,39	8,79	3169	0,68
6	40	<710	1,30	13,35	8,83	4849	0,45
7	20	<1000	1,29	13,43	8,89	2111	1,12
8	30	<1000	1,29	13,37	8,90	2919	1,10
9	40	<1000	1,26	13,32	8,69	4140	0,50

Color Values of Tablets

Table 4. documented the impact of PEG 4000 ratio and pomegranate peel particle size on the color values of the tablets. The results were consistent with those of the powder mixtures. As the tablets were manufactured by compressing the powder mixtures, similar outcomes were anticipated.

Table 4. Effect of PEG 4000 ratio and pomegranate peel powder particle size on the color of the tablets.

Sample #	PEG ratio (%)	Peel powder particle size (µm)	***L****	***a****	***b****
1	20	<500	57,10	12,43	25,76
2	30	<500	57,46	11,98	25,14
3	40	<500	58,55	11,54	24,54
4	20	<710	58,24	11,76	24,23
5	30	<710	58,78	10,43	21,88
6	40	<710	58,74	10,76	22,56
7	20	<1000	54,65	11,43	23,65
8	30	<1000	54,90	11,76	23,21
9	40	<1000	56,45	10,76	22,12

CONCLUSIONS

In conclusion, this study identified the potential use of pomegranate peel powder in the dietary supplement sector and established that the binder ratio and pomegranate peel particle size are significant factors in the production of dietary supplements. Powder blends of pomegranate peel powder and polyethylene glycol 4000 (PEG 4000) with low moisture content, good flowability, and low cohesiveness were successfully produced for tablet manufacturing. Considering the significant quality indicators like hardness and friability, it was concluded that the highest quality tablets were obtained using 40% PEG 4000 with pomegranate peel powder particle size of <710 µm. The findings will provide insights into sustainable use of pomegranate peel and its potential in developing antioxidant-rich supplements. The next part of the study can be to analyze the nutritional value of the tablets, emphasizing dietary fiber, phenolic content, antioxidant capacity, and bioacceptability of vitamins and other bioactive compounds.

REFERENCES

AACC, 2010. Approved methods of analysis (11th ed.), method 44-15.02 and method 08-01.01. ST. Paul, MN, USA. Cereals and Grains Association.

Alvarenga Botrel, D., Vilela Borges, S. 2012. Victória de Barros Fernandes, R.; Dantas Viana, A.; Maria Gomes da Costa, J.; Reginaldo Marques, G. Evaluation of spray drying conditions on properties of microencapsulated oregano essential oil. Int. J. Food Sci. Technol, 47(11): 2289-2296.

Aslani, A., Daliri, A. (2016). Design, formulation and evaluation of its physiochemical properties of acetaminophen, ibuprofen and caffeine as effervescent tablet. Journal of Reports in Pharmaceutical Sciences, 5(2), 122.

Atalay, D., Erge, H.S. 2018. Gıda Takviyeleri ve Sağlık Üzerine Etkileri. Food and Health, 4(2): 98-111.

Bushra, R., Shoaib, M. H., Aslam, N., Hashmat, D., Rehman, M. 2008. Formulation development and optimization of ibuprofen tablets by direct compression method. Pak. J. Pharm. Sci, 21(2): 113-120.

Canbağı, H., Uğurlu, T., Kerimoğlu, O. 2016. Çok Tabakalı Tablet Teknolojisi, Üretim Yöntemi, Değerlendirilmesi ve Kullanıldığı Çalışmalar. Marmara Pharmaceutical Journal, 20(3):380-389

Gandhi, A. 2012. Mouth dissolving tablets: a new venture in modern formulation technology. The pharma innovation, 1(8, Part A), 14.

Ho, L. H., Aziz, N. A. A., Azahari, B. 2013. Physico-chemical characteristics and sensory evaluation of wheat bread partially substituted with banana (Musa acuminata X balbisiana cv. Awak) pseudo-stem flour. Food Chemistry, 139: 532-539.

Jinapong, N., Suphantharika, M., Jamnong, P. 2008. Production of instant soymilk powders by ultrafiltration, spray drying and fluidized bed agglomeration. Journal of food engineering, 84(2), 194-205.

Jurenka, J. 2008. Therapeutic applications of pomegranate (Punica granatum L.): a review. Alternative medicine review, 13(2).

Kaderides, K., Kyriakoudi, A., Mourtzinos, I., Goula, A. M. 2021. Potential of pomegranate peel extract as a natural additive in foods. Trends in Food Science & Technology, 115, 380-390.

Kazaz A, Erdoğan M.G. 2020. Takviye edici gıda reklamlarında aldatıcı unsurlar. Gümüşhane Üniversitesi İletişim Fakültesi Elektron Derg., 8:930–60.

Koç, M., Koç, B., Kaymak-Ertekin, F. 2011. Toz gıdaların fiziksel karakterizasyon özellikleri. Akademik Gıda, 9(4): 60-70.

Kumar V, De La Luz Reus-Medina M, Yang D. 2002. Preparation, characterization, and tabletting properties of a new cellulose-based pharmaceutical aid. Int J Pharm. 235:129–40.

Kushwaha, S. C., Bera, M. B., Kumar, P. 2013. Nutritional composition of detanninated and fresh pomegranate peel powder. IOSR J. Environ. Sci. Toxicol. Food Technol, 7(1): 38-42.

Larhrib, H., Wells, J. I., Rubinstein, M. H. 1997. Compressing polyethylene glycols: the effect of compression pressure and speed. International journal of pharmaceutics, 147(2), 199-205.

Metiner, E. E., Bıyıklı, İ., Yücel, N., Türkoğlu, T., Dirim, S. 2023. Farklı kurutma yöntemleriyle meyve kabuğu tozu elde edilmesi: Fiziksel özellikler, toz ürün özelliği ve kurutma yöntemlerinin enerji verimliliği yönünden incelenmesi. Fırat Üniversitesi Fen Bilimleri Dergisi, 35(1): 29-37.

Ofori-Kwakye, K., Osei-Yeboah, F., Kipo, S. L. 2010. Formulation and quality evaluation of two conventional release tablet formulations. International Journal of Pharmaceutical Sciences Review and Research, 4(1): 94-99.

Osei-Yeboah F, Sun CC. 2015. Validation and applications of an expedited tablet friability method. Int J Pharm. 2015;484:146–55.

Rowayshed, G., Salama, A., Abul-Fadl, M., Akila-Hamza, S., Emad, A. M. 2013. Nutritional and chemical evaluation for pomegranate (Punica granatum L.) fruit peel and seeds powders by products. Middle East Journal of Applied Sciences, 3(4): 169-179.

Surek, E., Nilüfer-Erdil, D. 2016. Phenolic contents, antioxidant activities and potential bioaccessibilities of industrial pomegranate nectar processing wastes. International Journal of Food Science and Technology, 51: 231–239.

Sharma, K., Akansha, C. E. 2018. Comparative studies of proximate, mineral and phytochemical compositions of pomegranate (Punica granatum) in peel, seed and whole fruit powder. methods, 17: 18.

Taruna Syah, I., Darmadji, P., Pranoto, Y. 2016. Microencapsulation of refined liquid smoke using maltodextrin produced from broken rice starch. Journal of Food Processing and Preservation, 40(3): 437-446.

Topkaya C. 2012. Nar Kabuğu Tozu İlavesinin Keklerin Besinsel, Duyusal ve Mikrobiyolojik Özelliklerine Etkisi. Yüksek Lisans Tezi, Pamukkale Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, Denizli, Turkey.

Turchiuli, C., Fuchs, M., Bohin, M., Cuvelier, M. E., Ordonnaud, C., Peyrat-Maillard, M. N., Dumoulin, E. 2005. Oil encapsulation by spray drying and fluidised bed agglomeration. Innovative Food Science & Emerging Technologies, 6(1): 29-35.

Uca, E. 2019. Ultrafiltrasyon yöntemiyle Nar Kabuğundan fenolik bileşiklerin geri kazanılması ve sürecin optimizasyonu.Yüksek Lisans Tezi, Trakya Üniversitesi Fen Bilimleri Enstitüsü, Turkey.

Zhong, C., Zu, Y., Zhao, X., Li, Y., Ge, Y., Wu, W., ... Guo, D. 2016. Effect of superfine grinding on physicochemical and antioxidant properties of pomegranate peel. International Journal of Food Science & Technology, 51(1): 212-221.

INVESTIGATION OF MORPHOMETRIC VARIATIONS ON *PTEROCHLOROIDES PERSICAE* (CHOLODKOVSKY, 1898) (HEMIPTERA: APHIDIDAE) DEPENDS ON HOST PLANT PREFERENCES

Gizem BAŞER[1], Gazi GÖRÜR[1], Özhan ŞENOL[1]

[1] Niğde Omer Halisdemir University, Arts and Sciences Faculty, Biotechnology Department, Niğde, Türkiye
Corresponding author e-mail: gizem_baser@outlook.com

ABSTRACT

The brown peach aphid *Pterochloroides persicae* distributes in Southern Europe, North Africa, Southwest and Central Asia, Indonesia, Türkiye, India, and Pakistan. This aphid species cause serious damage to *Prunus* members (*Prunus persica, P. dulcis, P. domestica, P. armeniaca, P. salicina*). Although they show both holocyclic and anholocyclic life cycles, this species shows monoecious holocyclic life cycles in cooler climates. In recent years, it gradually widened its geographic distribution and prompted more financial destruction, becoming an important threat to peach and almond trees. So far, there is no study has been conducted in Türkiye with *P. persicae* related to its host plant preference or agricultural importance. The speciation process of *P.persicae* populations might occur based on their host plant preferences. In this context, this study aimed to find out morphometric variations of *P.persicae* depending on host plant usage. The study was conducted in Adıyaman, Malatya, Şanlıurfa, Afyonkarahisar, Kütahya, Uşak, Antalya, Muğla, Karaman, Erzurum, and Nigde provinces, and the samples collected from *Prunus* spp. The 25 morphometric characters were evaluated for statistical analyses from 228 aptera individuals of *P. persicae*. As a result of the study, 23 morphometric characters. It was determined that host plant preference plays an important role in morphological variations observed in *Pterochloroides persicae* populations.

Keywords: Morphological variation, *Prunus* spp, *Pterochloroides persicae*, Türkiye

INTRODUCTION

Aphids are obligate phytophagous insects that feed on plant sap. They have economic importance in terms of direct damage to the plant and indirectly as carriers of various plant viruses. Therefore, aphids are closely related to the host plant (Blackman and Eastop, 2023). Aphids show a great deal of phenotypic plasticity. Phenotypic plasticity has been shown to be very important for aphids to adapt to new host plants, to bring about new reproductive strategies and especially in the speciation of aphids (Görür, 2005). In aphids, the state of physiological and morphological characteristics of the host species, its locality, the effects of biotic and abiotic conditions may cause morphological differences in the same species. Studies have shown that aphids can show differences in morphological characters to adapt to environmental conditions (Hales et al., 2010; Siddiqui et al. 2019; Nibouche et al., 2021).

Brown peach aphid *Pterochloroides persicae* is a pest of *Prunus* spp. Although they show both holocyclic and anholocyclic life cycles depending on environmental conditions, this species shows monoecious holocyclic life cycles in colder climates (Talhouk, 1977; Blackman and Eastop, 2023). It is distributed in southern Europe, North Africa, south-west and central Asia, India, Pakistan and Indonesia and has recently increased its distribution and has become a significant threat to peach and almond trees in Romania and Tunisia (Blackman and Eastop, 2023). *P. persicae* has caused weakening of young fruit trees, drying of branches, reduction in yield and mould formation due to honeydew (Cross and Poswal, 1996; Moya, 2014; Mdellel, 2015). In addition, biotic and abiotic factors have caused changes in the growth and development period of *P. persicae* (Müller et al. 2001; Mdellel et al. 2011). The effect of host plant on the morphology of *P. persicae* has been reported in studies (Mdellel and Kamel, 2015; Mdellel et al, 2011).

In this study, it was aimed to reveal possible morphological differences due to different host preferences of *Pterochloroides persicae* feeding on *Prunus* spp. (*P. persica, P. dulcis, P. domestica, P. armeniaca, P. salicina).*

MATERIAL AND METHOD

Samples of *Pterochloroides persicae* preferring *Prunus persica, P. dulcis, P. domestica, P. armeniaca* and *P. salicina* host plants distributed in Adıyaman, Malatya, Şanlıurfa, Afyonkarahisar, Kütahya, Uşak, Antalya, Muğla, Karaman, Erzurum and Niğde provinces were performed. The samples were taken in eppendorf tubes containing 96 % ethyl alcohol and then prepared according to Martin (1983). After, the identification of the specimens was made according to the identification keys offered by Blackman and Eastop, 2023. Morphometric measurements of 3-4 wingless adult individuals suitable for morphometric analysis from each of the colonies in 5 different host plants were made under OLYMPUS BX51 brand microscope. Measurements of 25 morphological characters belonging to a total of 228 individuals were carried out. Measured characters are;

Body Length (BL), Body Width (BW), Total Antenna Length (AL), Antenna 1st segment length (A1L), Antenna 2 st segment length (A2L), Antenna 3 st segment length (A3L), Antenna 4 st segment length (A4L), Antenna 5 st segment length (A5L), Length of the 6 st Antenna Segment Processus Terminalis (A6PT), Length of the 6 st Antennal Segment Base (A6BASE), Length of segments IV and V of the rostrum (URSL), Rostrum segment IV width (URSW), Cauda length (CL), Caud Width (CW), Diameter of Siphunculi (SIP BD), Hind tarsus I. segment length (HTI), Hind tarsus II. segment length (HTII), Hind Femur Length (HFL), Hind Femur Width (HFW), Fore Femur Length (FFL), Fore Femur Width (FFW), Hind Tibia Length (HTL), Longest hair length of antennal 3 st segment (A3HL), Antenna 5 primary rhinaria width (A5RW), Antenna 6 primary rhinaria width (A6RW)

Canonical Analysis of Variance (CVA) was performed to determine the principal components of variation in morphological data. One-way Analysis of Variance (ANOVA) and Multiple Comparison Analysis (Tukey-HSD Test) were evaluated to determine the possible effects of the host plant on the morphological characteristics of *Pterochloroides persicae* members. SPSS ver 26.0 package programme was used for statistical analyses.

RESULTS AND DISCUSSION

Measurements of 25 morphological characters of 228 individuals of *Pterochloroides persicae* collected from 5 different host plants (*Prunus persica, P. dulcis, P. domestica, P. armeniaca, P. salicina*) were carried out.

Morphometric variations depending on the host plant

As a result of the evaluation of the obtained morphological characters, it was determined that the measured characters of the population sampled in the *Prunus armeniaca* host were shorter than other populations characters. These variations among *P. persicae* populations were tested by applying One-Way Analysis of Variance (ANOVA). In 22 of the 25 characters measured, it was observed that the host plant caused statistically significant differences on *P. persicae* populations (Table 1).

Following the differences detected by applying ANOVA, Multiple Comparison Analysis (Tukey-HSD test) was performed. Especially, morphological features of the *Pterochloroides persicae* population collected on *Prunus armeniaca* differed from other populations. In addition to the overall differences in BL, there are also differences between *P. armeniaca* and *P. dulcis* (Tukey HSD[37.72]=-0.34, P=0.010), differences in BW between *P. armeniaca* and *P. domestica* populations (Tukey HSD[37.72]=-0.25, P=0.023), differences in AL between *P. armeniaca* and *P. salicina* (Tukey HSD[35.91]=-0,11, P=0.008) respectively. Moreover, there are differences in HTI between *P. armeniaca* and *P. dulcis* populations (Tukey HSD[34.63]=-0,00, P=0.001) and differences in HFL between *P. armeniaca* and *P. persica* (Tukey HSD[35.67]=-0,14, P=0.007). Furthermore, Canonical Vector obtained according to Wilk's lambda analysis is significant with P=0.00 and P=0.11 values and Function 1 (CV1) accounts for 39.5% of the variances; Function 2 (CV2) explains 35% of variances indicating strong host plant effects on morphological features of the *P. persicae* populations. Standardised Canonical Analysis of Variance (CVA) was also performed to determine the relative significance of the characters used to separate the populations related with host plant usage. According to Canonical Vector 1 (CV1), the highest values belong to HFL (1,381), FFL (0,988), HFW (0,842); according to Canonical Vector 2 (CV2), the highest values belong to AL (1,058), A3L (0,604) and A5L (0,689).

Table 1. Differences among morphological characters (ANOVA) of *Pterochloroides persicae* populations collected on different host plants, *Prunus* spp. ($P < 0.05$)

	Sum of Squares			
	Between groups	**Within groups**	**F**	**P**
BL	5,958	59,887	5,547	,000
BW	1,689	34,936	2,696	,032
AL	,436	3,191	6,998	,000
A1L	,002	,031	2,665	,033
A2L	,001	,007	4,947	,001
A3L	,067	1,163	3,030	,019
A4L	,015	,133	6,035	,000
A5L	,021	,087	12,008	,000
A6BASE	,002	,033	3,252	,013
URSL	,002	,034	2,849	,025
CL	,004	,074	2,955	,021
SIPBD	,372	1,839	11,133	,000
HTI	,001	,008	6,203	,000
HTII	,012	,059	10,759	,000
FFL	,262	2,448	5,709	,000
FFW	,015	,097	8,149	,000
HFL	1,064	7,753	7,202	,000
HFW	,019	,094	10,632	,000
HTL	4,616	28,115	8,538	,000
A3HL	,000	,005	3,621	,007
A5RW	,001	,007	3,713	,006
A6RW	,001	,005	7,054	,000

The host plant influences on the morphometric characters was given graphically by discriminant function analysis. It was observed that *P. persisae* populations differed in morphometric characters depending on host plant preference, especially *P. armeniaca* clearly separated from others (Figure 1).

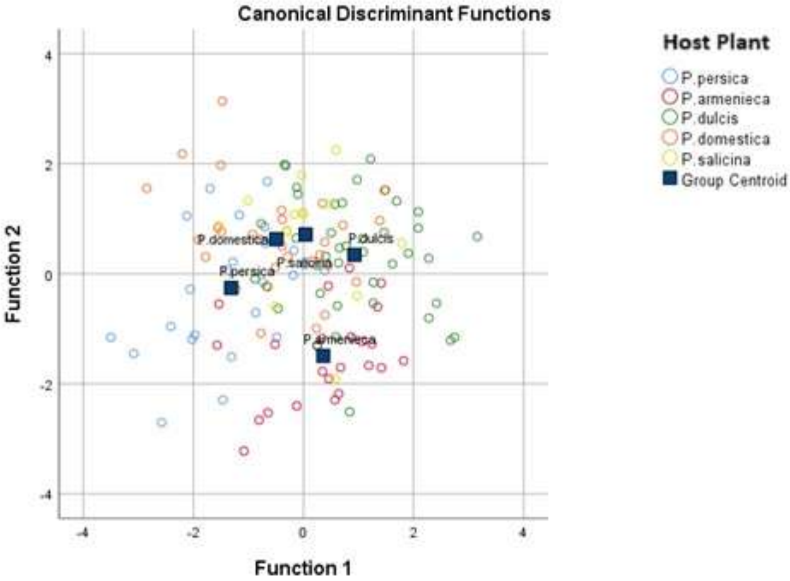


Figure 1. Classification of *Pterochloroides persicae* populations according to canonical discriminant function depending on host plant

CONCLUSIONS

In this study, *Pterochloroides persicae* populations feeding on 5 different host plants (*P. persica, P. armeniaca, P. dulcis, P. domestica, P. salicina*) were evaluated to reveal morphometric variations related with the host plant utilization. According to the results of ANOVA analyses, there are significant differences in 23 morphometric characters of the P. persicae populations sampled from 5 different host plants, *Prunus* spp. Hind femur length, total antenna length, fore femur length, hind femur width, antenna 3 and 5 length were the most differentiated characters in the differentiation of *P. persicae* populations on *Prunus* spp. Among 23 measured characters, 9 of the morphometric characters were incorporated for the first time when comparing *P. persicae* populations collected from different host plants. Findings of the presented study shown similar host plant effects with the previous studies. Adouani et al. (2021) measured 16 morphometric characters and among these characters, body length, body width, total antennal length, hind femur length showed a significant difference. Mdellel and Kamel (2015), 13 morphological characters were determined by ANOVA. Among them, there were significant differences in the length of antenna segments I, IV and V, body length and siphunculi diameter. Mdellel and Kamel (2015a), measured 12 morphometric characters and showed differences in 1 antennal segment, body length and cauda length. The host plant appears to play an effective role in the morphology of the *P. persicae* population. This study is the first morphometric study on *P. persicae* populations, which is one of the important agricultural pests in Türkiye.

ACKNOWLEDGMENTS

Authors thank to TUBITAK (Project number:115Z325, 119Z250) for partial support of the study.

REFERENCES

Adouani, R. B., Mdellel, L., Kamel, M. B. H., Martinez, D. T. 2021. Morphology, genetics and biology of Pterochloroides persicae Cholodkovsky (Hemiptera: Lachninae) and their effect on Pauesia antennata Mukerji (Hymenoptera: Aphidinae) behaviour. *African Entomology*, *29*(1), 59-68.

Blackman, R. L., Eastop, V. F. 2023. Aphids of the world's plants. An online identification and information guide, http://www.aphidsonworldsplants.info.

Cross, A. E., Poswal, M. A. 1996. Dossier on PauesiaantennataMukerji. Biological Control Agent for the Brown Paech Aphid, Pterochloroides persicae in Yemen, International Institute of Biological Control.

Görür, G. 2005. The importance of phenotypic plasticity in herbivorous insect speciation. In Insect Phenotypic Plasticity,153-180. CRC Press.

Hales, D., Foottit, R. G., Maw, E. 2010. Morphometric studies of the genus Sitobion Mordvilko 1914 in Australia (Hemiptera: Aphididae), *Australian Journal of Entomology***,** 49(4), 341-353.

Martin, J.H. 1983. The identification of common aphid pests of tropical agriculture, *Tropical Pest Management***,** 29, 395-411.

Mdellel, L., Kamel, M. B. H., Da Silva, J. A. T. 2011. Effect of Host Plant and Temperature on Biology and Population Growth of Pterochloroides persicae Cholodv (Hemiptera: Lachninae). *Pest technology*, *5*(1), 74-78.

Mdellel, L., Kamel, M. B. H. 2015. Effect of host plant on morphology of Pterochloroides persicae Cholodkovsky 1899 (Hemiptera, Aphididae), *J. Entomol. Zool. Stud*, 3, 324-327.

Mdellel, L., Kamel, M. B. H. 2015a. Morphometry and biological parameters of different instars of the giant brown peach aphid: Pterochloroides persicae Cholodkovsky 1899 (Hemiptera: Aphididae) in Tunisia. In Annales de la Société entomologique de France (NS), Taylor & Francis, 51(1), 4-9.

Moya, L. 2014. Pterochloroides Persicae, Una plaga de reciente aparición, pulgón negro de las ramas.

Müller, C. B., Williams, I. S., Hardie, J. 2001. The role of nutrition, crowding and interspecific interactions in the development of winged aphids, *Ecological Entomology*, 26(3), 330-340.

Nibouche, S., Costet, L., Medina, R.F., Holt, J.R., Sadeyen, J., Zoogones, A-S., Brown, P., Blackman, R.L. 2021. Morphometric and molecular discrimination of the sugarcane aphid, Melanaphis sacchari, (Zehntner, 1897) and the sorghum aphid Melanaphis sorghi (Theobald, 1904), *PLoS ONE*,16(3).

Siddiqui, J.A., Zou, X., Liu, Q., Zhang, H., Lin, X., Huang, X. 2019. Functional Morphology and Defensive Behavior in a Social Aphid, *Insects*, 10(6), 163.

Talhouk, A. S. 1977. Contribution to the knowledge of almond pests in East Mediterranean countries. VI. The sap sucking pest, Zeitschrift für Angewandte Entomologie 83, 248–25.

COMPARISON OF THE ANTIBACTERIAL EFFECTS OF SOME SOLVENTS OF COMMERCIAL ASTAXANTHIN ON *AEROMONAS HYDROPHILA*

Halil İbrahim KÜÇÜKER[1,], Aycan ULUTAŞ[2], Jale KORUN[3]*

[1] *Yılmaz İş Makineleri, Evka-3 mah., 129/6 sok. No:11, 4. Sanayi sitesi, Bornova-İzmir, Türkiye*
[2] *Kılıç Deniz Ür. AŞ Didim Şubesi Bafa Su Ürünleri Yavru Balık Üretim Tesisi, Aydın, Türkiye*
[3]*Akdeniz Üniversity, Faculty of Fisheries, Department of Fish Diseases, Antalya, Türkiye*
Corresponding author e-mail: jalekorun@akdeniz.edu.tr
**In this study, a part of H. İ. Küçüker's Master's Science Thesis is summarized.*

ABSTRACT

Aeromonas hydrophila is an important fish pathogen and causes serious economic losses especially in freshwater and brackish water under culture conditions. The aim of the present study was to determine the antibacterial effect of astaxanthin on the strains of *A. hydrophila*. The MIC values of astaxanthin were compared using different solvents. These included ethanol, water, and 2-propanol. Sick rainbow trout (*Oncorhynchus mykiss*) samples used in the study were obtained from a commercial trout farm. Body weights of the fish varied between 56 g and 225 g. After external findings observed in the fish were recorded, necropsy was performed on the fish and the findings detected in the internal organs were recorded. For bacteriological studies, liver, spleen, and kidney were inoculated onto petri dishes containing Brain Heart Infusion Agar (BHIA). After 72 h of incubation at 24 ± 2 °C, subcultures were made from the bacterial colonies that grown in the dishes. According to the morphological, physiological, and biochemical test results and API 20E rapid diagnostic kit results, 6 strains were identified as *A. hydrophila*. In the study, the commercial form of astaxanthin was used for the determination of its antibacterial effect. For this purpose, astaxanthin solutions were prepared using water, ethanol, and 2-propanol. The value at which bacterial growth was not observed was evaluated as Minimal Inhibition Concentration (MIC). According to the results of the study, it was determined that the solution of astaxanthin prepared with 2-propanol was effective on *A. hydrophila*, while the solution prepared with water did not have effective.

Keywords: *Aeromonas hydrophila*, Rainbow trout, *Oncorhynchus mykiss*, Astaxanthin, Antibacterial effect.

INTRODUCTION

Aquaculture is on a continuous growth trend worldwide to fulfil the global for edible fish. This growth can cause fish to be kept in intensive production, resulting in severe stress that can adversely affect the development, growth, and immune systems of fish, and subsequently various disease outbreaks (Korni and Khalil, 2017, Preena et al., 2021). Bacterial diseases, which are among the infectious diseases, cause low growth rate, low meat quality, morbidity/mortality, and reduce fish production.This situation results in a decrease in the profit, margins of fish farms and as a result, significant economic loss for aquaculture sector (Jeeva et al., 2013, Salem et al., 2020, Yassen et al., 2022).

Motile *Aeromonas* septicemia (MAS) is a bacterial infection caused by certain species of the genus *Aeromonas*, mainly *Aeromonas hydrophila* (Preena et al., 2021, Yassen et al., 2022). The disease is commonly observed in fish species and can result in high mortality rates (Korni and Khalil, 2017, Abraham and Bardhan, 2019). Fish species affected by MAS include edible fish such as Nile tilapia (*Oreochromis niloticus*), rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) and channel catfish (*Ictalurus punctatus*) (Ashraf et al., 2018, Cao et al., 2020, Dien et al., 2023) and ornamental fish species such as guppy (*Poecilia reticulata*), zebrafish (*Danio rerio*), and goldfish (*Carassius auratus*) (Perreta et al., 2018; Hossain et al., 2019, 2020). Clinical signs associated with *Aeromonas* infections include exophthalmos, hemorrhage, ulceration, fin rot, lethargy, scale loss, skin discoloration, and hemorrhagic/necrotic viscera. In some cases, acute mortality has been observed, with fish dying rapidly before developing symptoms of the disease (Kumar et al., 2016, Azzam-Sayati et al., 2021).

Antibiotic treatment is used in human medicine as well as in the treatment and control of bacterial infections (Stratev and Odeyemi, 2017). With the expansion of aquaculture worldwide, the use of antibiotics for the treatment of bacterial infections as well as the use of antibiotics for prophylactic purposes has increased (Stratev and Odeyemi, 2017, Scarano et al., 2018). The Multiple Antibiotic Resistance (MAR) has been reported in *A. hydrophila* strains (Le et al., 2018). This resistance can be attributed to the misuse and overuse of antibiotics and to the genetic adaptability of *A. hydrophila* (Hossain and Heo, 2020) and occurs through conjugation, mutation, or horizontal gene transfer in the natural environment (Watts et al., 2017). Indeed, antimicrobial resistance in *A. hydrophila* is often mediated by genes on the bacterial chromosome, plasmids or integrons that confer resistance to most beta-lactam antimicrobials (Abdulhasan et al., 2019). The emergence of antimicrobial resistance in *A. hydrophila* poses a significant challenge to the effective treatment of infections caused by this bacterium. This situation also highlights the need for responsible use of antibiotics and non-antibiotic infection control measures to prevent the further spread of drug-resistant strains (Stratev and Odeyemi, 2017). Therefore, alternative methods have been developed to prevent and/or treat *A. hydrophila* infections. These methods include probiotics, vaccination, and phytobiotics (Stratev and Odeyemi, 2017).

Astaxanthin (3,3´-dihydroxy-β, β-carotone-4, 4´-dione) was discovered in 1970 by the English chemist Professor Basil Churles Weeden. Astaxanthin is a naturally occurring carotenoid found in marine organisms such as microalgae, salmon, trout, krill, shrimp, crabs, and crustaceans. The green microalgae, *Haematococcus pluvialis* is a rich source of astaxanthin. *Chlorella zofingiensis*, *Chlolococcum* spp. and other microalgae such as *Botryococcus braunii* contain astaxanthin (Köksal, 2008). Carotenoids are a class of bioactive natural products synthesized exclusively by plants, phytoplankton, algae, bacteria, and some fungal species (Lim et al., 2018, Brotosudarma et al., 2020). Due to factors such as the high chemical diversity of carotenoids and the host protection of some carotenoids from oxidation and related damage, scientific and commercial interest in carotenoids has increased in recent years (Galasso et al., 2018, Brotosudarma et al., 2020).

Astaxanthin is the most important carotenoid used in aquaculture (Lim et al., 2018). This important carotenoid pigment is used to make rainbow trout and salmon flesh more reddish (Lim et al., 2018). Other effects of astaxanthin that make it important aquaculture are that it is a growth promoter and can increase the survival rate and antioxidant capacity of fish (Saleh et al., 2018). Saleh et al. (2018) found an increase in some immune parameters as well as a decrease in the effects of stress factors in sea bass (*Dicentrarchus labrax*) fed with astaxanthin-supplemented diets. Tizkar et al. (2015) reported an increase in sperm concentration in goldfish (*C. auratus*) fed with 150 mg/kg astaxanthin-supplemented diets.

The aim of this study was to determine the antibacterial effects of astaxanthin, used in the coloring of rainbow trout carcasses in Türkiye, on *A. hydrophila* strains. The MIC values of astaxanthin using different solvents including ethanol, water, and 2-propanol were compared.

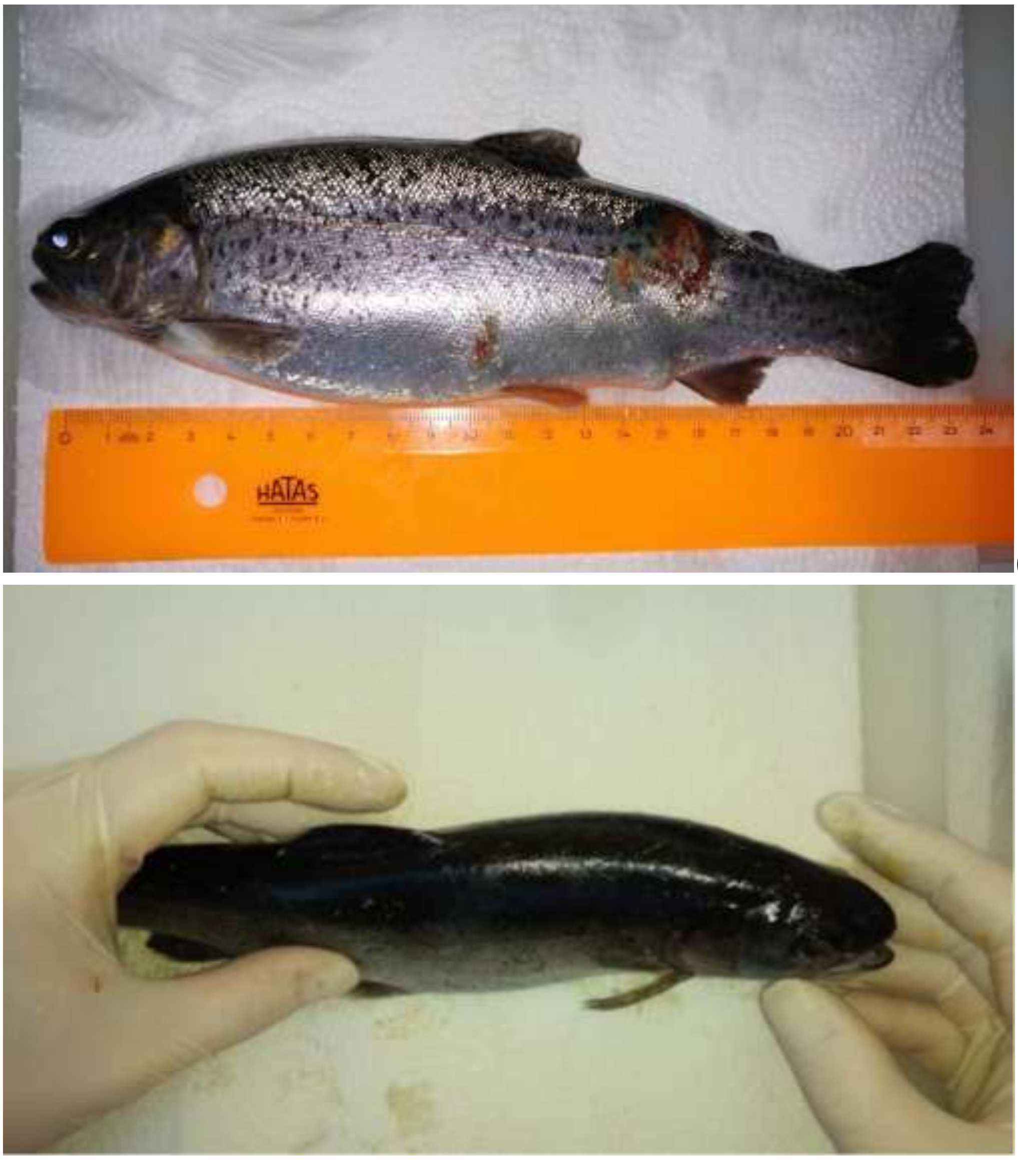
MATERIAL AND METHOD

The diseased fish samples (from 56 to 225 g) used in the study were obtained from commercial trout farms in Antalya (Türkiye) and necropsy was performed on the fish. Samples of liver, spleen, and kidney from the fish were inoculated on Brain Heart Infusion Agar (BHIA) for bacteriological analysis. All plates were incubated at 24 ± 2 °C for 72 h (Austin and Austin, 2012). The necessary permissions were obtained from the local ethics committee for animal experiments of Akdeniz University (744/2018.03.003). After the incubation period at 24 ± 2 °C for 72 h, the morphology and color of the bacterial colonies on BHIA plates were examined and then subcultures of the isolates were made. A series of physiological and biochemical tests including cytochrome oxidase, catalase, O/F (Leifson) fermentation, H_2S production, growth at different temperature degrees, growth in different salt concentrations, methyl red and Voges-Proskauer (VP), indole production citrate utilization, starch hydrolysis, NO_2 production, acid production from sugars and growth on MacConkey agar (Austin and Austin, 2012). The API 20E kit (BioMerieux, France) was also used for the identification of strains isolated from diseased fish. The kit was prepared according to the manufacturer's instructions and it was incubated at 24 ± 2 °C for 24-48 h. At the end of the incubation period, the reagents were added to the kit and the results were read using the APIWEB identification software.

The antibacterial effects of astaxanthin were determined by using the macro dilution method. For this purpose, solutions of astaxanthin were prepared with ethanol, water, and 2-propanol. Commercial astaxanthin powders (Roche) of 1 mg, 0.5 mg, and 0.1 mg were used in the study. For 1 mg/ml stock solution, 10 mg of astaxanthin was dissolved in 10 ml of ethanol, water, and 2-propanol. For 0.5 mg/ml stock solution, 10 mg astaxanthin was dissolved in 20 ml ethanol, water, and 2-propanol. For 0.1 mg/ml stock solution, 10 mg astaxanthin was dissolved in 100 ml of ethanol, water, and 2-porpanol (Erdağlı, 2011). The stock solutions were sterilized before use by means of 0.2 µm disposable membrane filters. Then 1 ml of cation-adjusted Mueller-Hinton broth (CMHB) (CLSI, 2006) was added to each tube. Starting from the first tube, twofold dilutions were made, and the inoculated tubes were incubated at 24 ± 2 °C for 16-24 h. At the end of the incubation period, it was determined from which tube bacterial growth started and the micro values of astaxanthin were determined in terms of % concentrations. The lowest concentration at which turbidity was not observed was taken as the minimum inhibitory concentration (MIC) for that strain. The inoculated bacterial solution was used as control.

RESULTS AND DISCUSSION

In the study, behavioral findings such as lethargy, swimming near to the water surface, loss of appetite, and reduced feed intake were detected in sick fish. Exophthalmos, abdominal dropsy, loss of scales, and skin lesions (Figure 1a), darkening of skin color (Figure 1b), paleness of gills, adhesion and melting at the ends of gill filaments, hemorrhages under the jaw and on the fins were observed.



(a)

(b)

Figure 1. (a) Abdominal dropsy, loss of scales and skin lesions caused by *Aeromonas hydrophila* (b) Darkening of the skin in sick fish

At necropsy, hemorrhage in the mouth and muscle tissue, pyloric seka, paleness of the liver (Figure 2), swelling and hemorrhage in the air bladder, splenomegaly and melting of the kidneys were detected.

Figure 2. Paleness of the liver, hemorrhages in pyloric seka and muscle in sick fish

Fish infected with *A. hydrophila* may show symptoms of loss of appetite, pallor of the gills and skin ulcers. Gills, kidney, liver, spleen, pancreas, and skeletal muscle are among the organs frequently affected by the disease (Swam and White, 2014). The findings observed in the disease depend on the virulence of the bacterium, the resistance of the fish to infection, the presence or absence of bacteremia or septicemia and other stress factors related to fish (Swan and White, 2014). Wamala et al. (2018) reported that *A. hydrophila* isolated from Nile tilapia (*O. niloticus*) and African catfish (*Clarias gariepinus*) caused hemorrhages and ulcers on the skin, exophthalmos, swollen abdomen, and acidic fluid accumulation in the abdomen. In addition, fin rot was observed in tilapia, while mortalities were observed in both species. Laith and Najiah (2013) informed lethargy, shallow hemorrhagic ulcers as well as deep ulcers exposing muscle tissue in *C. gariepinus* naturally infected with *A. hydrophila*. In some fish, hemorrhages were observed at the base of the fins and in the anus. At necropsy, pale liver and gills enlarged kidney and accumulation of yellowish fluid in the body cavity was also detected. Timur et al. (2010) reported that *A. hydrophila* was isolated from young Russian sturgeon (*Acipenser gueldenstaedtii*) cultured in concrete ponds in 2002-2003 as a single causative species or as a mixed infection with *Flavobacterium hydatis* and reported that these disease agents caused bacterial hemorrhagic septicemia in fish, resulting in low mortality. In the present study, lethargy, decreased feed intake, swimming near to the water surface, darkening of the skin color, exophthalmos, abdominal dropsy, pallor of the gills, skin lesions and loss of scales in sick fish were observed and these findings were like the other researchers' findings (Timur et al., 2010; Laith and Najiah, 2014; Wamala et al., 2018).

As a result of bacteriological studies, cream-colored, motile, fermentative, cytochrome oxidase and catalase positive, rod-shaped six bacterial strains were isolated from the liver, spleen, and kidney of the sick fish. The strains were indole-producing, arginine dihydrolase positive and hydrolyzed starch. The strains grew on MacConkey agar. The biochemical properties of the strains are given in Table 1.

Table 1. Biochemical test results of *A. hydrophila* strains

Tests	Strains (N=6 strains)	Abbott et al., (2003) (N=25 strains)
Motility	+	+
Cytochrome oxidase	+	+
Catalase	+	+
Indole	+	96% positive
Urea	-	-
Citrate	+	88% positive
VP	-	92% positive
LDC	+	+
ADH	+	+
Acid from:		
Lactose	+	16% positive
Mannose	+	+
Sorbitol	+	-
Inositol	+	-
Sucrose	+	+
Arabinose	+	94% positive
Mannitol	+	96% positive
Gelatine	-	96% positive
NaCl (0%) growth	+	+
NaCl (3%)	+	+

+: positive result -: negative results

The API 20E kit results were evaluated by adding reagents at the end of the 24-48 h incubation period at 24 ± 2 °C. According to the APIWEB identification software, 6 strains isolated from sick fish were identified as *A. hydrophila*. The results of the API 20E rapid diagnostic kit are shown in Table 2.

Table 2. API 20E diagnostic kit results

Tests	Reactions/Enzymes	Results
ONPG	B-galactosidase	+
ADH	Arginine dihydrolase	+
LDC	Lysine decarboxylase	+
ODC	Ornithine decarboxylase	-
CIT	Citrate utilization	-
H_2S	H_2S production	-
URE	Urease	-
TDA	Tryptophan deaminase	-
IND	Indole production	+
VP	Acetoin production	+
GEL	Gelatinase	+
GLU	Fermentation/oxidation (Glucose)	+
MAN	Fermentation/oxidation (Mannitol)	+
INO	Fermentation/oxidation (Inositol)	-
SOR	Fermentation/oxidation (Sorbitol)	-
RHA	Fermentation/oxidation (Rhamnose)	-
SAC	Fermentation/oxidation (Saccarose)	+
MEL	Fermentation/oxidation (Melibiose)	-
AMY	Fermentation/oxidation (Amygdalin)	+
ARA	Fermentation/oxidation (Arabinose)	-
OX	Cytochrome oxidase	+
Nitrate reduction	NO_2 production	+
N_2	Reduction to N_2 gas	-
MOB	Motility	+
McC	Mac Conkey medium growth	+
OF-F	Fermentation	+
OF-O	Oxidation	+

+: positive, -: negative

Nahar et al. (2016) used API 20E rapid diagnostic kit to identify 10 *A. hydrophila* strains isolated in their study. The researchers reported that the strains reacted positively to beta-galactosidase, arginine dihydrolase, lysine decarboxylase, sodium citrate, urea, tryptophan deaminase, sodium pyruvate, gelatinase, glucose, mannitol, sucrose, melibiose, amygdalin, and arabinose. However, the test results of ornithine decarboxylase, sorbitol, and rhamnose were negative. The *A. hydrophila* isolated in our study were found to be positive for beta-galactosidase, arginine dihydrolase, glucose, mannitol, amygdalin in the API 20E kit, but urea production, arabinose, and TDA results were found to be different from the results reported by Nahar et al. (2016). In the present study, ornithine decarboxylase, sorbitol, and rhamnose test results of the strains were found to be as negative. These results agreed with the results reported by Nahar et al. (2016).

MIC values were determined using solutions of astaxanthin prepared with different solvents. It was determined that astaxanthin did not have antibacterial activity against *A. hydrophila* when water was used as solvent. However, the solution prepared with 2-propanol was more antibacterial activity than the solution prepared with ethanol. Also, 7.8125 µg/ml, 15.625 µg/ml and 31.25 µg/ml concentrations of ethanol did not have antibacterial effect on the strains. It was determined that there was not bacterial growth at concentrations ranging from 62.5 µg/ml to 500 µg/ml. In the study, it was found that 2-propanol was not effective on strains at 7.8125 µg/ml, while it was effective at 62.5 µg/ml and 500 µg/ml.

Poor water quality, inadequate feed, and a suppressed immune system contribute to infection by opportunistic fish pathogens such as *A. hydrophila* and antimicrobials are often used to treat and control bacterial diseases (Mahanty et al., 2013). However, antimicrobials used to treat bacterial fish diseases may result in the emergence of drug resistance bacterial strains (Harikrishnan and Balasundaran, 2005). This situation has led researchers to search for alternatives to antibiotics. These alternatives include prebiotics, probiotics as well as the use of medical and local plants (Bhuvaneswari and Balasundaram, 2006; Tareq et al., 2014). In the present study, solutions of astaxanthin prepared with different solvents were used on *A. hydrophila* strains isolated from sick rainbow trout. According to the test results, it was found that water was not effective, but 2-propanol was effective.

CONCLUSIONS

Aeromonas hydrophila is one of the most important bacterial infectious agents affecting the aquaculture of marine, ornamental, and freshwater including rainbow trout. Until today, antimicrobial drugs have been used in the treatment of bacterial fish diseases. Intensive and random use of drugs may lead to the development of resistance to these drugs in bacterial species. For this reason, the research and use of alternatives to antimicrobial agents, especially antibiotics, has come to the spotlight. Today, more than one study is being carried out on this subject and solutions are being tried. Astaxanthin is known to have strong antioxidant properties. In this study, it was investigated *in vitro* whether astaxanthin has antibacterial properties as an alternative to antibiotics on *A. hydrophila* strains, which is frequently isolated in cases of motile *Aeromonas* septicemia caused by stress in rainbow trout farms. For this purpose, the antibacterial effect of different solvents of astaxanthin prepared with water, ethanol, and 2-propanol on *A. hydrophila* was studied and it was found that among the solutions of astaxanthin prepared with different solvents, especially 2-propanol was effective while water was ineffective. The present study is a basic study for astaxanthin. Therefore, it is thought that the results of the *in vitro* study of astaxanthin may be the basis of for further *in vivo* studies.

REFERENCES

Abbott, S. L., Cheung, W. K. W., Janda, J. M. 2003. The genus *Aeromonas*: Biochemical characteristics, atypical reactions, and phenotypic identification schemes. Journal of Clinical Microbiology, 41(6): 2348-2357.

Abdulhasan, S. A., Alattar, N. S., Jaddoa, N. T. M. 2019. Comparative study of some virulence factors and analysis of phylogenetic tree by 16S rDNA sequencing of *Aeromonas hydrophila* isolated from clinical and environmental samples. Journal of Sciences, 60(11): 2390-2397.

Abraham, T. J., Bardhan, A. 2019. Emergence and spread of antimicrobial resistance in motile aeromonads of the aquaculture environment. Indian Journal of Animal Health, 58(2): 39-52.

Ashraf, A., El-Tawab, A., Saleh, O. A., Awad, S. M., Absi, H. M. 2018. Phenotypic and genomic characters on *Aeromonas* species causing mortality in fishes. Benha Veterinary Medical Journal, 35(1): 285-300.

Austin, B., Austin, D. A. 2012. Bacterial Fish Pathogens. Diseases of farmed and Wild Fish. Springer, UK.

Azzam-Sayati, M., Ina-Salwany, Md. Y., Zamri-Saad, M., Yusof, M. T., Annas, S., Nahihah, Md. Y., Liles, M. R., Monir, Md. S., Zaidi, S., Amal, M. N. A. 2021. The prevalence, putative virulence genes and antibiotic resistance profiles of *Aeromonas* spp. Isolated from cultured freshwater fishes in peninsular Malaysia. Aquaculture, 540:1-12.

Bhuvaneswari, R., Balasundaram, C. 2006. Antibiotic resistance of *Aeromonas hydrophila* isolated from *Piaractus mesopotamicus* (Holmberg, 1887) and *Oreochromis niloticus* (Linnaeus, 1758). Scientia Agricola, 63(3): 81-84.

Brotosudarma, T. H. P., Limantara, L., Setiyono, E., H. 2020. Structures of astaxanthin and their consequences for therapeutic application. International Journal of Food Science. 2156582, 1-16.

Cao, Y., Li, S., Han, S., Wang, D., Zhao, J., Xu, L., Liu, H., Lu, T. 2020. Characterization and application of a novel *Aeromonas* bacteriophage as treatment for pathogenic *Aeromonas hydrophila* infection in rainbow trout. Aquaculture, 70: 1-8.

CLSI (Clinical and Laboratory Standards Institute), 2006. Methods for antimicrobial disk susceptibility testing of bacteria isolated from aquatic animals, approved guideline. CLSI document M42-A, 26(23), Clinical and Laboratory Standards Institute, Wayne, USA.

Dien, L. T., Ngo, T. P. H., Nguyen, T. V., Kayansamruaj, P., Salin, K. R., Mohan, C. V., Rodkhum, C., Dong, H. T. 2023. Non-antibiotic approaches to combat motile *Aeromonas* infection in aquaculture: current state of knowledge and future perspectives. Reviews in Aquaculture, 15: 333-365.

Erdağlı, I. 2011. Investigation of antimicrobial effect of capsaicin on *Staphylococcus aureus* and *Enterococcus faecalis*. Department of Pharmaceutical Microbiology, Erciyes University, Faculty of Pharmacy, Kayseri, Türkiye, 53 p.

Galasso, C., Orefice, I., Pellone, P., Cirini, P., Miele, R., Ianora, A., Brunet, C., Sansone, C. 2018. On the neuroprotective role of astaxanthin: new perspectives? Marine Drugs, 16(247): 1-16.

Harikrishman, R., Balasundaran, O. 2005. Modern trends in *Aeromonas hydrophila* disease management with fish. Reviews in Fisheries Sciences, 13: 281-320.

Hossain, S., Heo, G. I. 2020. Ornamental fish: a potential source of pathogenic and multidrug-resistant motile *Aeromonas* spp. Letters in Applied Microbiology, 72: 2-12.

Hossain, S., Dahanayake, P. S., De Silva, B. C. J., Wickramanayake, M. V. K. S., Wimalasena, S. H. M. P., Heo, G. J. 2019. Multidrug resistant *Aeromonas* spp. Isolated from zebrafish (*Danio rerio*): antibiogram, antimicrobial resistance genes and class 1 integron gene cassettes. Letters in Applied Microbiology, 68: 370-377.

Hossain, S., De Silva, B. C. J., Dahanayake, P. S., De Zoysa, M., Heo, G. J. 2020. Phylogenetic characteristics, virulence properties and antibiogram profile of motile *Aeromonas* spp. Isolated from ornamental guppy (*Poecilia reticulata*). Archives of Microbiology, 202: 501-509.

Jeeva, S., Packia Lekshimi, N. C., Rja Brindha, J., Vasudevan, A. 2013. Studies on antibiotic susceptibility of *Aeromonas hydrophila* isolated from goldfish (*Carassius auratus*). International Journal of Current Microbiology and Applied Sciences, 23: 1492-1499.

Korni, F. M. M., Khalil, F. 2017. Effect of ginger and its nanoparticles on growth performance, cognition capability, immunity, and prevention of motile *Aeromonas* septicaemia in *Cyprinus carpio* fingerlings. Aquaculture Nutrition, 23: 1492-1499.

Köksal, M. 2008. The effect of different environmental conditions (light, temperature, nutrient deficiency and ventilation) on growth and astaxanthin content in *Haemotococcus pluvialis* Flatow. Çukurova University, Institute of Science and Technology, Master's Thesis, 52 pages.

Kumar, K., Pande, V., Singh, L., Sharma, L., Soxena, N., Thakuria, D., Singh, A. K., Sahoo, P. K. 2016. Pathological findings of experimental *Aeromonas hydrophila* infection in golden mahseer (*Tor putitara*). Fisheries and Aquaculture Journal, 7: 160.

Laith, A. R., Najiah, M. 2014. *Aeromonas hydrophila*: antimicrobial susceptibility and histopathology of isolates from diseased catfish, *Clarias gariepinus* (Burchell). Journal of Aquaculture Research and Development, 5 (2): 1-7.

Le, T. S., Nguyan, T. H., Vo, H. P., Doan, V. C., Nguyan, H. L., Tran, M. T., Tran, T. T., Southgate, P. C., Kurtböke, D. I. 2018. Protective effects of bacteriophages against

Aeromonas hydrophila causing motile Aeromonas septicemia (MAS) in striped catfish. Antibiotics, 7(16): 1-11.

Lim, K. C., Yusoff, F. Md., Shariff, M., Kamarudin, M. S. 2018. Astaxanthin as feed supplement in aquatic animals. Reviews in Aquaculture, 10: 738-773.

Mahanty, A., Mishra, S., Bosu, K., Maurya, V. K., Netam, S. P., Sarkar, B. 2013. Phytoextracts-synthesized silver nanoparticles inhibit bacterial fish pathogen *Aeromonas hydrophila*. Indian Journal of Microbiology, 53(4): 438-446.

Nahar, S., Rahman, M. M., Ahmed, G. V., Faruk, A. R. 2016. Isolation, identification, and characterization of *Aeromonas hydrophila* from juvenile farmed pangasius (*Pangasianodon hypophthamus*). International Journal of Fisheries and Aquatic Sciences, 4(4): 52-60.

Perreta, A., Antúnez, K., Zunino, P. 2018. Phenotypic, molecular, and pathological characterization of motile aeromonads isolated from diseased fishes cultured in Uruguay. Journal of Fish Diseases, 41: 1559-1569.

Preena, P. G., Dharmaratnam, A., Kumar, V. J. R., Swaminathan, T. R. 2021. Plasmid-mediated antimicrobial resistance in motile aeromonads from diseased Nile tilapia (*Oreochromis niloticus*). Aquaculture Research, 52: 237-248.

Saleh, N. E., Wassef, E. A., Shalaby, S. M. 2018. The role of dietary astaxanthin in European sea bass (*Dicentrarchus labrax*) growth, immunity, antioxidant competence and stress tolerance. Egyptian Journal of Aquaculture Biology and Fisheries, 22(5), 189-200.

Salem, M., Zharan, E., Saad, R., Zaki, V. 2020. Prevalence, molecular characterization, virulotyping, and antibiotic resistance of motile aeromonads isolated from Nile tilapia farms at Northern Egypt. Mansaura Veterinary Medical Journal, 21(1): 56-57.

Scarano, C., Piras, F., Virdis, S., Zino, G., Nuvoloni, R., Dalmasso, A., De Santis, E. P. L., Spanu, C. 2018. Antibiotic resistance of *Aeromonas* spp. strains isolated from *Sparus aurata* reared in Italian mariculture farms. International Journal of Food Microbiology, 284: 91-97.

Stratev, D., Odeyemi, O. A. 2017. An overview of motile *Aeromonas* septicemia management. Aquaculture International, 25: 1095-1105.

Tareq, U. Z. M., Chowdhury, M. B. R., Dipu, M. R. 2014. Isolation of bacterial fish pathogen *Aeromonas hydrophila* and therapeutic effects of medicinal plants on its invasion. Journal of Fisheries, 2(1): 76-79.

Timur, G., Akaylı, T., Korun, J., Yardımcı, R. E. 2010. A study on bacterial hemorrhagic septicemia in farmed young Russian sturgeon (*Acipenser gueldenstaedtii*) in Turkey. Istanbul Universitesi Su ürünleri Dergisi, 25(1): 19-27.

Tizkar, B., Kazemi, R., Alipour, A., Seidavi, A., Naseralavi, G., Ponce-Palafox, J. T. 2015. Effects of dietary supplementation with astaxanthin and β-carotene on the semen quality of goldfish (*Carassius auratus*). Theriogenology, 84: 1111-1117.

Wamala, S. P., Mugimba, K. K., Mutoloki, S., Evensen Ø., Mdegela, R., Byaruga, D. K., Sørum, H. 2018. Occurrence and antibiotic susceptibility of fish bacteria isolated from *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African catfish) in Uganda. Fisheries and Aquatic Sciences, 21:6.

Watts, J. E. M., Schreier, H. J., Lanska, L., Hale, M. S. 2017. The rising tide of antimicrobial resistance in aquaculture sources: sources, sinks and solutions. Marine Drugs, 18: 158-174.

Yassen, D. A., El-Gawad, E. A. A. A., El-Asely, A. M., Elabd, H. S., Shaheen, A. A., 2022. Control of motile *Aeromonas* septicemia in Nile tilapia (*Oreochromis niloticus*) using some herbal additives. Benha Veterinary Medical Journal, 42: 180-185.

PROSPECTS OF USE OF BUFFALO BREED BULGARIAN MURRAH IN UKRAINE

Igor Goncharenko

National University of Life and Environmental Sciences of Ukraine. Kyiv
igorgoncharenko2711@gmail.com

The development of buffalo breeding is extremely promising for Ukrainian agriculture. In particular, the prospects are good for the dairy industry: Murrah buffaloes have better milk yields than other buffalo breeds. Buffaloes are quite durable and cheap in feed consumption. They are highly resistant to blood-borne diseases. They have satisfactory slaughter qualities - 48-55% meat yield. Indicators of fat, protein and dry matter content in buffalo milk show us that it can become the basic basis for children's nutrition and production of food products of high biological quality, which are highly valued abroad. The meat productivity of Murrah buffaloes is also quite high. We have an excellent example of using this breed of buffalo in Bulgaria, which gives us a fair right to repeat their success, adapting it to the realities of the Ukrainian agricultural market. Thus, all these advantages can be used to improve the level of the export market, which will also have a positive impact on Ukraine's economic potential. To do this, a number of changes should be made. In particular, it is necessary to create scientific and production breeding and genetic state associations for buffalo breeding in Ukraine, it is necessary to separate buffaloes from the cattle population into a separate group, to set a standard and a wholesale price for buffalo products. In addition, it is necessary to create special programs to preserve the buffalo gene pool, with the allocation of appropriate state funding and attraction of foreign investment. The perspectives for the use of Bulgarian Murrah buffaloes in Ukraine are very high, but only if appropriate steps are taken. Otherwise, unfortunately, we should expect t he gradual extinction of buffaloes in Ukraine, and along with them the potential to improve the condition of a rather important part of our agricultural industry.

Key words: buffalo breeding, Bulgarian Murra, buffalo selection in Ukraine

INTRODUCTION

The use of buffalos in Bulgaria has its origins in their economic activities. Since the beginning of the twentieth century, buffaloes have been used for agricultural work, in particular, as a source of meat, as well as draft animals: they have a speed of 3 km/h and can pull a load of 900-1500 kg. According to A. Agabeyli, the number of buffaloes in Bulgaria at the beginning of the twentieth century was 500 thousand [1].

During the Soviet period, Ukraine did not attribute due importance to the prospects of using these animals in agriculture; they were not even separated from the cattle population. There was also no special standard and no wholesale price for buffalo products. These problems have not been addressed in independent Ukraine either. Today, we can observe the irrational use of very powerful cattle in farms of various forms of ownership, the lack of an appropriate buffalo register and common standards for their valuation in Ukraine.

An excellent example of the use of buffaloes is their selection and breeding in Bulgaria. The Bulgarian breeders in 1962 asked themselves how they could further improve the efficiency of using these animals, in particular their milk production? The solution was to import Indian sires of the high-yielding Murrah breed. This was the beginning of the creation of a new dairy breed, the Bulgarian Murrah. Coordination of the breeding of the Bulgarian Murrah breed was carried out under the scientific guidance of the Buffalo Research Institute in Shumen and the National Center for Animal Breeding. In order to optimize the genetic improvement of the Bulgarian buffalo population, to increase milk production, selectors have developed measures and programs to improve the Bulgarian buffalo, and introduced artificial insemination. As a result of these measures, the type of local Bulgarian buffalo has evolved into the dairy type of the Murrah buffalo. Today in Bulgaria 80% of the buffaloes belong to this breed and most of the animals are raised in individual farms [4]. What are the advantages of Murrah buffalo breeding and what should we implement in Ukrainian agriculture today?

RESEARCH RESULTS

The Murrah buffalo is a large animal, with a live weight of 800-950 kg for males and 500-780 kg for females (see picture). The duration of pregnancy is 290-340 days. The average age at first calving is between 32 and 40 months. The inter-calving period is 436 to 505 days, depending on the genotype, feeding and keeping conditions.

Under satisfactory feeding and housing conditions, buffaloes are inseminated at 17-20 months, although under optimal conditions, economic maturity occurs at 10-12 months. The animals are long-lived - some live up to 60 years, even at the age of 45-55 years a buffalo can mate and give birth to viable offspring, however they are kept until 25-30 years of age. Murrah buffaloes have well-developed, rounded udders, so they are well suited for machine milking. Additional evidence of the high genetic potential of Bulgarian Murrah buffaloes for high quality milk production is that many buffaloes have milk yields above 2500-3000 kg, and some of them produce more than 4500 kg of milk per lactation. The highest milk yields were obtained from F2 crosses - 5349 kg of milk with a fat content of 6.64% for 305 days of lactation [5].

At the same time, the milk productivity of other buffalo breeds used in Ukraine is lower, at about 1500 liters of milk per 270-280 days of lactation, so Murrah buffaloes are more productive for dairy products. It should be noted that the production of buffalo milk is cheaper than cow's milk.

Fig. Buffaloes of the Bulgarian Murrah breed in the indoor and outdoor environment

On average, it contains 7-8% fat (6.5-10.5) and 4.3-5.1% protein, up to 23% dry matter, and up to 5.1% lactose. Buffalo milk is homogenized abroad to improve its quality and taste, and fermented milk products such as mazzoni, cream, butter, and mozzarella cheese have excellent taste and are in great demand in the area where they are bred, and therefore can become a very important attribute of the Ukrainian export market.

The main source of buffalo meat is bulls. Studies conducted on the growing qualities of young buffaloes show significant differences compared to conventional cattle. The average daily weight gain of young buffaloes is 650-1083 g. The most effective slaughter weight is recognized as a live weight of 400 kg.

Murrah buffalo meat is used to produce excellent raw dried products, which mature 10-11 days faster than other cattle meat.

Another advantage of Murrah buffaloes is their digestive system, which is much larger than that of other cattle (the length of the intestine is 31 times longer than the length of the body). The most intensive absorption of nutrients in Murrah buffaloes takes place in the small intestine (55.8-62.5%), which indicates a high level of feed digestibility. They usually consume cheap, rough fodder, including fern, quinoa, nettles, burdocks, sedge, ordinary corn stalks without preparation for feeding, cereal and legume straw, branch fodder and fodder from marsh vegetation. During the summer, buffaloes mainly eat only foot fodder, and occasionally their owners feed them [2, 3].

Thus, the development of buffalo breeding is extremely promising for Ukrainian agriculture. In particular, the prospects are good for the dairy industry: Murrah buffaloes have better milk yields than other buffalo breeds. Buffaloes are quite durable and cheap in feed consumption. They are highly resistant to blood-borne diseases. They have satisfactory slaughter qualities - 48-55% meat yield. Indicators of fat, protein and dry matter content in buffalo milk show us that it can become the basic basis for children's nutrition and production of food products of high biological quality, which are highly valued abroad. The meat productivity of Murrah buffaloes is also quite high. We have an excellent example of using this breed of buffalo in Bulgaria, which gives us a fair right to repeat their success, adapting it to the realities of the Ukrainian agricultural market.

CONCLUSION

Thus, all these advantages can be used to improve the level of the export market, which will also have a positive impact on Ukraine's economic potential. To do this, a number of changes should be made. In particular, it is necessary to create scientific and production breeding and genetic state associations for buffalo breeding in Ukraine, it is necessary to separate buffaloes from the cattle population into a separate group, to set a standard and a wholesale price for buffalo products. In addition, it is necessary to create special programs to preserve the buffalo gene pool, with the allocation of appropriate state funding and attraction of foreign investment. The perspectives for the use of Bulgarian Murrah buffaloes in Ukraine are very high, but only if appropriate steps are taken. Otherwise, unfortunately, we should expect t he gradual extinction of buffaloes in Ukraine, and along with them the potential to improve the condition of a rather important part of our agricultural industry.

REFERENCES

1. Гузеев Ю.В., Гончаренко И.В., Винничук Д.Т. (2016). Современное развитие буйволоводства: цифры и факты. Вісник Сумського НАУ. Серія "Тваринництво". *Modern development of buffalo farming: figures and facts. Bulletin of Sumy NAU. Series "Livestock".* 7 (30). 62-69.

2. Гузєєв Ю.В., Гончаренко І.В., Вінничук Д.Т. (2014). Перспективи розвитку буйволоводства у світі та в Україні. Зоотехнічна наука: історія, проблеми, перспективи. *Prospects for the development of buffalo breeding in the world and in Ukraine. Animal science: history, problems, prospects*. 12-14.
3. Гузєєв Ю.В. Сокуренко О.І Демчук М.П. (2001). Генофонд буйволів України. Науковий вісник Національного університету біоресурсів і природокористування. *Gene pool of buffalos in Ukraine. Scientific newsletter of the National University of Biological Resources and Environmental Sciences*. 160/1. 291-294.
4. Захариев З., Алексиев А., Пеева Ц. (1986). БИВОЛИ. България 247.
5. Пеева Ц. Българска мурра. Породи селскостопански животни в България . Каталог. 216.

THE EFFECT OF DİFFERENT RATES OF MUSHROOM COMPOST ON THE HEAVY METAL (Al, Cd, Co, Cr, Ni, Pb) CONTENT OF PERENNIAL RYEGRASS PLANTS

Özlem ETE AYDEMİR[1], Mehmet AKGÜN[2], Faruk ÖZKUTLU[1]

[1] Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ordu University, 52200, Ordu, Türkiye
[2]Hazelnut Specialization Coordinatorship, Giresun University, 28100, Giresun, Türkiye

Corresponding author: ozlemete87@gmail.com

ABSTRACT

The use of waste mushroom compost (MC) in the establishment of lawns can have a positive effect on reducing the heavy metal content of turfgrass and MC can effectively immobilize the uptake of heavy metals by plants. The waste mushroom compost can affect the heavy metal content in perennial ryegrass grown by mixing different ratios into soils. In this study, the use possibilities of mushroom waste compost for lawn establishment were evaluated. The research was established in the randomized block design in the form of a pot trial in field conditions in Ordu province. Mushroom compost (MC) and soil (S) were mixed in various rates (100% S, 75% S + 25% MC, 50% S + 50% MC, 75% S + 25% MC and 100% MC) and applied as cover material after sowing. In the experiment, dry perennial ryegrass yield and aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), nickel (Ni) and lead (Pb) contents of perennial ryegrass plants were determined. According to the results, it was determined that 50% S + 50% MC mixture was more effective on the dry herbage yield of perennial ryegrass plants in cover materials prepared by mixing mushroom compost with soil. The application of cover materials prepared at different rates caused differences in Al, Cd, Co, Cr, Ni and Pb contents of perennial ryegrass plants. Heavy metal contents of perennial ryegrass plants grown in 100% MC application increased compared to 100%S treatment (control). Al, Cr and Pb contents decreased by 4-fold, 2.5-fold and 3.5-fold, respectively, when perennial ryegrass was grown in 50% Soil + 50% mushroom compost mixture compared to 100%S (control) treatment. In this mixture, Cd content increased to 0.07 mg kg^{-1} from 0.05 mg kg^{-1} in the control. Similarly, Ni content increased from 0.91 to 1.51 mg kg^{-1}. In our results, it is recommended that waste mushroom compost should be applied as a mixture with soil since the use of 100% of waste mushroom compost as a cover material in the creation of perennial ryegrass area causes a decrease in dry perennial ryegrass yield and an increase in heavy metal content.

INTRODUCTION

Heavy metals such as cadmium and lead can accumulate in agricultural soils through industrial activities, mining and contaminated fertilizer. After entering the soil, heavy metals can be taken up by plants, potentially entering the food chain and posing a risk to human and animal health (Kabata-Pendias, 2011). To reduce soil pollution, organic-based media such as mushroom compost can be used as soil amendments. This by-product of mushroom cultivation can increase soil fertility and improve plant health by reducing heavy metal bioavailability (Amir et al., 2005). Adding compost to the soil can also influence heavy metal accumulation in perennial ryegrass plants. Compost can improve the physical and chemical properties of the soil, leading to perennial ryegrass growth and higher heavy metal accumulation in the plant. The addition of compost can enhance microbial activity in the soil, which can increase the degradation of organic pollutants and increase the solubility of mineral nutrients, making them more accessible to the plant. The addition of substances of organic origin to the soil can increase the cation exchange capacity (CEC) of the soil, which can lead to immobilization of heavy metals and decrease their uptake by plants (Bolan et al., 2003). Benitez et al. (2005) found that the addition of mushroom compost to a heavy metal contaminated soil reduced the bioavailability of Cd, Pb and zinc (Zn) and reduced their uptake by ryegrass (*Lolium perenne* L.). Similarly, Amir et al. (2005) reported that application of mushroom compost to a Pb-contaminated soil reduced Pb concentration in shoots and roots of ryegrass. The tolerance of plants to heavy metals is impacted by several physiological and biochemical processes, involving the production of antioxidants and the activation of stress response pathways. There is limited information on the specific effects of mushroom compost on the uptake of Al, Cd, Co, Cr, Ni and Pb by perennial ryegrass. The aim of this research was to investigate the effects on heavy metal content of perennial ryegrass grown in soil with the application of different proportions of mushroom compost and to determine the optimum proportion of mushroom compost to maintain acceptable growth and biomass production and to reduce the uptake of heavy metals.

MATERIAL AND METHOD

The research was carried out in Ordu University Research and Application land under field conditions in baton type pots numbered 12. The waste mushroom compost used in the experiment was obtained from Antalya-Korkuteli Gülat mushroom compost company. Perennial ryegrass (*Lolium perenne* L.) was used as grass plant in the experiment. The results of the analyses of some properties of the soil (Table 1) and waste mushroom compost (Table 2) are given. In experiment, air dry 6 kg of soil sieved through a 4 mm sieve was added to each pot. After the mushroom compost added to the pots, each pot was equalized to 8 kg. The experiment was carried out according to the random plots experimental design with 3 replications.

Table 1. Some physical and chemical properties of the soil used in the experiment

Soil properties												
Texture	EC	pH	$CaCO_3$	O. M.	P	K	Ca	Mg	Fe	Cu	Mn	Zn
	dS m^{-1}	(1:2.5)	%		mg kg^{-1}							
loamy	0.11	5.3	0.96	2.07	20	86	3740	1050	9.71	0.3	8.68	0.13

O.M: Organic Matter

Table 2. Some chemical properties of the waste mushroom used in the experiment

Waste Mushroom Properties									
pH	EC	Total N	C	C/N	Total P	Total K	Total Ca	Total Mg	Total B
	dS m^{-1}	%			%				mg kg^{-1}
7.04	5.89	2.18	33.75	16.4	0.85	2.68	5.91	1.2	32.8
Total Fe	Total Cu	Total Mn	Total Zn	Al	Cd	Co	Cr	Ni	Pb
mg kg^{-1}									
5995	61	489	354	4355	1.11	7.89	39.74	111	2.18

Plants Growth

After sowing in pots, the pots were watered with 60-70% of the water in the field capacities of the soils when needed until harvesting under open field conditions. The harvest of green parts of the perennial ryegrass was determined depending on the level of growth retardation. Accordingly, the plants were harvested 1 cm above the soil level before flowering. Harvested plants were washed with pure water and dried at 65 ^{0}C for 48 hours. Dry weights of the dried plants were determined, and dry matter yields were determined.

Soil and Plant Analyses

Texture of the soil used in the experiment: According to hydrometer method (Bouyocous, 1951); Soil pH (Soil Reaction) using soil/water 1:2.5 ratio, determination of EC in Soil: Total salt by EC-meter (Richards, 1954) in 1:2.5 soil/water ratio suspension. Lime ($CaCO_3$) measurement using 1:3 HCl/water ratio (Allison & Moodie 1965). Organic matter Walkley & Black (1934); Plant-available phosphorus Bray & Kurtz (1945). Plant-available potassium, calcium and magnesium were determined according to Pratt (1965); Plant-available iron, copper, manganese and zinc were determined according to Lindsay & Norvell (1978).

For the determination of mineral elements in perennial ryegrass samples, 0.25 g of the plant samples were weighed and burnt in a muffle furnace at 550 °C to ash. Then, it was boiled with 10 N HNO_3 (2 ml), and it was completed to 50 ml with pure water and filtered through Whatman blue band filter paper. Cd, Co, Cr and Pb were determined by ICP-OES (Perkin Elmer 2100V).

All data used the means among treatments were compared with excell. Results were given in the form of mean±std.

RESULT AND DISCUSSION

Dry Grass Yield

It was determined that there were differences in the effects on dry perennial ryegrass yield with the application of waste mushroom compost at different ratios as a cover material in perennial ryegrass field formation. While the dry perennial ryegrass yield was 165 kg da^{-1} in 100% soil application, it increased approximately 2.5 fold in 50% S + 50% MC environment and was obtained as 364 kg da^{-1}. Under 100% waste mushroom compost application alone, it was determined that 83 kg dry perennial ryegrass yield was obtained per decare with a 2-fold decrease (Table 3). The use of waste mushroom compost (MC) as a soil amendment can significantly increase the dry matter yield of perennial ryegrass plants in turf areas. Several studies have shown the beneficial effects of MC on the growth and productivity of various perennial ryegrass species. In a study by Jankowski et al., (2018) it was found that supplementing MC with minerals increased the biomass yield of lawn perennial ryegrass in turf areas. In addition, MC can increase soil organic matter content, improve soil structure, and improve water retention capacity. The use of waste mushroom compost is a promising approach to increase the dry matter yield and quality of perennial ryegrass plants in lawn fields. Its nutrient-rich composition and ability to improve soil properties make it an attractive alternative to synthetic fertilizers. However, it has been shown that the use of waste mushroom compost alone is not sufficient for hay yield and quality, and it shows that better results can be obtained with mixtures with soil in certain proportions.

Tablo 3. Effect of mushroom compost application on perennial ryegrass yield (kg da^{-1})

Treatments	Perennial Ryegrass Yield		
100% S	165	±	22
75% S+25% M.C	313	±	74
50% S+50% M.C	364	±	68
25% S+75% M.C	346	±	50
100% M.C	83	±	37

S: Soil, M.C: Mushroom Compost

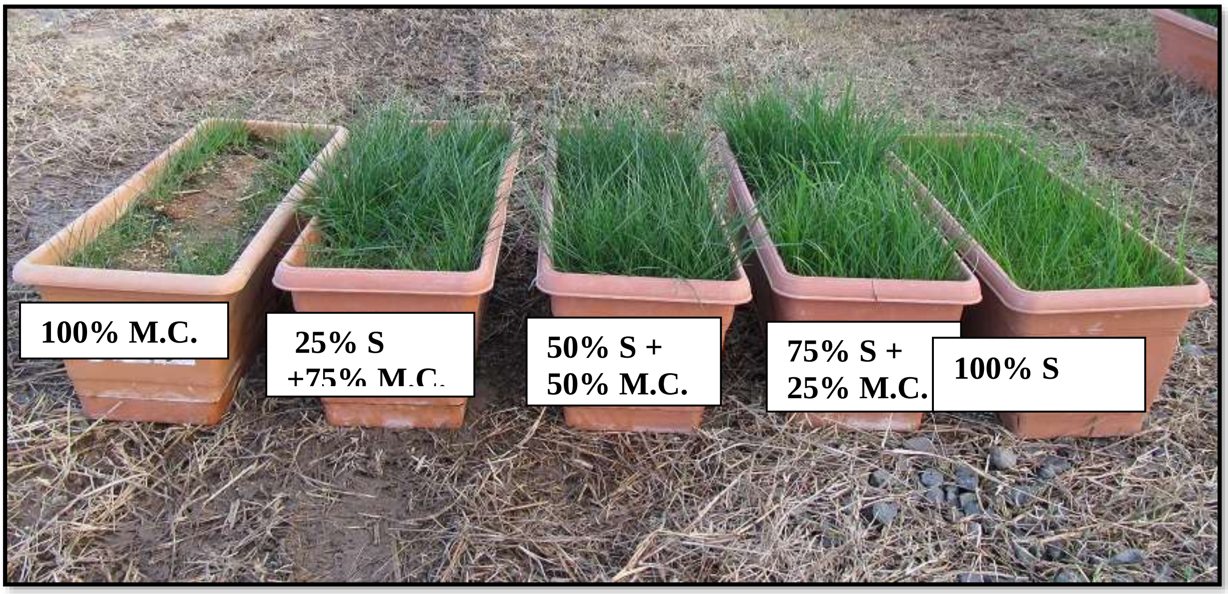


Figure 1: The effect of different doses of mushroom compost on the yield of perennial ryegrass plants.

Al, Cd, Co, Cr, Ni and Pb Content of Perennial Ryegrass (*Lolium perenne* L.) Grown with the Application of Waste Mushroom Compost

As seen in Table 4, it has been determined that there are significant differences in the heavy metal contents of grass plants grown by applying Waste Mushroom compost in different environments with soil. While the Al content of the grass plant in 100% soil condition was 777 mg kg^{-1}, it increased to 1232 mg kg^{-1} in 100% MC application. Similar trend, it was determined that this increase also occurred in the heavy metals Cd, Co, Cr, Ni and Pb. As shown in Table 4, it was determined that there was a significant decrease in Al, Co, Cr and Pb contents in grass plants grown under 50% soil + 50% mushroom mixture in 5 different environments.

Table 4. Al, Cd, Co, Cr, Ni and Pb content of perennial ryegrass (mg kg^{-1})

Treatments	Al			Cd			Co			Cr			Ni			Pb		
100% S	777	±	31	0.05	±	0.02	0.51	±	0.20	2.84	±	0.72	0.91	±	0.23	1.81	±	0.49
75% S+25% M.C	333	±	95	0.10	±	0.02	0.67	±	0.40	1.69	±	0.34	2.20	±	0.91	0.63	±	0.12
50% S+50% M.C	184	±	10	0.07	±	0.02	0.34	±	0.13	1.12	±	0.36	1.51	±	0.29	0.51	±	0.06
25% S+75% M.C	382	±	71	0.05	±	0.01	0.36	±	0.06	1.89	±	0.93	1.23	±	0.11	0.87	±	0.50
100% M.C	1232	±	116	0.06	±	0.04	1.08	±	0.67	4.30	±	0.13	3.30	±	0.68	2.24	±	0.33

Applying compost can affect heavy metal uptake in grass. Several studies have found that compost application generally does not lead to significant increases in heavy metal concentrations in grass or other crops. One study found that compost application "did not cause any phytoxicity to crops" and that yields were not markedly affected by the heavy metals supplied by the compost. Another study concluded that "the compost slightly increased heavy metal concentrations in the soil but did not cause any phytoxicity to crops (Zhang et al., 2000; Zając & Skrajna, 2024). This effect is due to the type and composition of the compost used significantly affecting its effectiveness in immobilizing heavy metals. Composts amended with materials such as biochar, bentonite, waste mushroom compost or other mineral additives have been shown to exhibit a significant and improved ability to bind heavy metals and reduce their bioavailability (Huang et al., 2016). Apart from these, the organic matter, pH and cation exchange capacity of the compost play an important role in retaining heavy metals. Another study that is consistent with our results was explained by Zając & Skrajna (2024) that application of waste mushroom compost such as MSC did not lead to high Al, Co, Cr or Pb levels in grass plants such as *Miscanthus sinensis*. The low heavy metal content of the heavy metal flour itself in growing grass plants with compost applications is, in a way, due to the low content of the compost used. If the waste mushroom compost used contains low metal content, we can think that it is unlikely to pose a risk of heavy metal pollution in lawns. However, it is important to note that specific heavy metal levels may vary depending on the source and composition of mushroom compost and further research may be needed to confirm these findings across different compost sources and grass types.

CONLUSUION

According to available evidence from this research, application of waste mushroom compost alone increases the uptake of heavy metals such as Al, Cd, Co, Cr, Ni and Pb by perennial ryegrass grown under it. It shows that a mixture of 50% soil and 50% waste mushroom compost can be an effective strategy to reduce heavy metal contents. The ability of S+MC to immobilize organic matter and nutrient content as well as heavy metals through adsorption and complexation processes contribute to this beneficial effect. Further research is needed to fully understand the mechanisms underlying the effects of S+MC on heavy metal availability and plant uptake, as well as to investigate the long-term implications of this approach for sustainable land management and phytoremediation.

REFERENCES

Allison, L. E., Moodie, C. D. 1965. Carbonate. Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties, 9, 1379-1396.

Amir, S., Hafidi, M. Merlina, G. Hamdi, H. Revel, J. C. Hafidi, M. 2005. Fate of Polycyclic Aromatic Hydrocarbons During Composting of Lagooning Sewage Sludge. Chemosphere, 58(4): 449-458.

Benitez, E., Romero, E. Gomez, M. Gallardo-Lara, F. Nogales, R. 2005. Bioremoval of Heavy Metals From Urban Composts by Eisenia fetida. Bioresource Technology, 96(16), 1783-1787.

Bolan, N. S., Adriano, D. C. Curtin, D. 2003. Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability. Advances in Agronomy, 78: 215-272.

Bouyoucos, G. J. 1951. A recalibration of the hydrometer method for making mechanical analysis of soils.

Bray, R. H., Kurtz, L. T. 1945. Determination of Total Organic and Available Forms of Phosphorus in Soils. Soil Sci 45:39–45. https://doi.org/ 10.1097/00010694-194501000-00006

Huang, M., Zhu, Y. Li, Z. Huang, B. Luo, N. Liu, C. Zeng, G. 2016. Compost as a Soil Amendment to Remediate Heavy Metal-Contaminated Agricultural Soil: Mechanisms, Efficacy, Problems, and Strategies. Water, Air, & Soil Pollution, 227:1-18.

Jankowski, K., Malinowska, E. Sosnowski, J. Wiśniewska-Kadżajan, B. Kaczorek, A. 2018. Effects of Spent Mushroom Substrate and Slurry on Nutritional Value of Grass and Medicago x Varia T. Martyn Mixtures. International Journal of Agricultural and Biological Engineering, 11(3): 61-66.

Kabata-Pendias, A. 2011. Trace elements in soils and plants (4th ed.). CRC Press.

Lindsay WL, Norvell WA (1978) Development of a DTPA Soil Test For Zinc, Iron, Manganese and Copper. Soil Sci. Soc Am J 42:421–428. https://doi.org/10.2136/sssaj1978.03615995004200030009x

Pratt, P. F. 1965. Potassium methods of soil analysis. (Editor: C. A. Black) part-2. Agron. Series No:9: 1010-1022 Am. Soc. of Agron., Inc. Madison, Wisconsin, USA.

Richards, L. A. 1954. Diagnosis and improvement of saline and alkali soils. United States Depatyment of Agriculture Handbook, vol 60

Walkley, A. Black, I.A. 1934. An examination of the Degtjareff Method For Determining Soil Organic Matter, and A Proposed Modification of The Chromic Acid Titration Method. Soil Sci. 37:29-38.

Zając, M., Skrajna, T. 2024. Effect of Composted Organic Waste on Miscanthus sinensis Andersson Energy Value. *Energies*, *17*(11), 2532.

Zhang, M., Heaney, D. Solberg, E. Heriquez, B. 2000. The effect of MSW compost on metal uptake and yield of wheat, barley and canola in less productive farming soils of Alberta. Compost Science & Utilization, 8(3):224-235.

CONSERVATION STATUS OF ALBANIA COASTAL DUNE HABITATS (92/43/EEC HABITATS DIRECTIVE)

Prof. Assoc. Dr. Alma Imeri, Prof. Dr. Alfred Mullaj[2]*
** Department of Plant science, Faculty of Agricultural and Environment, Agricultural University of Tirana, Albania. Street "Pajsi Vodica", Koder-Kamez, Tirane. Albania.*
aimeri@ubt.edu.al *(corresponding author)*
[2] University of Tirana, Faculty of Nature and Science, Department of Biology, Avenue "Zogu i pare", Tirane, Albania.
amullaj@fshn.edu.al

ABSTRACT

Coastal dunes are among habitats with the worst conservation status on a global. Reporting theirs loss due to strategic importance of them is our duties protect the unique biodiversity heritage of the Albania. In this study we will try to figure up the costal vegetation. Highlighting its updated current conservation status at the EFD. The conservation status was evaluated through the following criteria: geographic range, surface area, structure, functions, pressures, threats, conservation measures and prospects. We have collected more than 100 releves in Albania shoreline, (mainly in Adriatic coast). Focused on the floristic study, tota plants identified 220 species and their endangerment status, based on red book as well as phytosociological study. The first group comprises of the *Cakilo-Xanthietum*, and *Eryngio-Sporoboletum.* The second group compose stable dunes with *Euphorbio paraliae-Agropyretum junceiformis, Medicagini marinae-Ammophiletum australis* and *Ephedra distachya* communities Results highlighted the dramatically bad conservation status of Albanian coastal dunes. Results showed a generalized threat and an alarming conservation status both on herbaceous and wooded communities, in particular in some relevant habitats, such as the embryo dunes. Plants associations are classified based on principles of Zurich–Montpellier school that made the bas of classification in Europe sites. Main pressures and threats were linked to residential, commercial and industrial activities, as well as alien species. Although some of the changes in distribution and trends are probably deriving from more accurate and updated data, the alarming conservation status of Albania coastal areas requires a better knowledge of pressures and threats for further management actions and monitoring plans. Finally, this scientific paper will be a powerful instrument for the future management of Adriatic costal habitats from a conservation perspective.

Key words: *Coastal dunes, EFD, embryo dunes, conservation status.*

INTRODUCTION

Coastal dunes are complex ecosystems occupying zones of transition between terrestrial and marine ecosystems. (Urban Silk 2016). This is a very dynamic environment, with distinct degrees of stabilization depending on the topography, natural disturbance and distance from the sea, and it harbors a mosaic of habitats (van der Maarel 2003). Plant communities on sand dunes have specific ecological requirements determining their position along the gradient from the sea inland (Carboni et al. 2009). Various ecological factors influence the distribution of plants along this gradient: sand abrasion, salt spray, burial by sand, erosion, accretion, tide level, wave attack, wind blasting and dehydration (Maun 2009; Miller et al. 2010). Zonation is very stable and a regular sequence of plant communities along the gradient can be observed worldwide (Doing 1985; Attorre et al. 2013). Vegetation of beaches and mobile dunes are considered azonal (Doing 1985), while more inland, stable dunes are covered by plant communities more adapted to local conditions (Buffa et al. 2012). Sand dune vegetation occurs all along the Mediterranean coasts and several authors have provided syntaxonomical schemes of this vegetation type. In the central and eastern part of the Mediterranean, overviews have been made for Italy (Géhu et al. 1984; Géhu & Biondi 1996; Brullo et al. 2001; Biondi 2007; Pirone 2014), Greece (Sýkora et al. 2003) and Turkey (Géhu et al. 1989), but not for Adriatic coast along the Balkan Peninsula. Research on psammophytic (sand dunes) vegetation started with Beck von Mannagetta (1901) and Morton (1915), who refer to the "Formation des Dünensandes". Horvatić (1934, 1939) was the first to study and provide a deeper insight into this vegetation type according to the Braun-Blanquet method, on the islands of Pag and Rab (Croatia), and he added new data in 1963 (Horvatić 1963). Extensive studies in Croatia were carried out by Trinajstić (1974, 1989a, 1995), Trinajstić & Jasprica (1998) and Alegro et al. (2003, 2004) Trinajstić & Jasprica (1998) and in Montenegro by Trinajstić (1989b) and Mijović et al. (2006, 2012). Vegetation research in Albania applying the Braun-Blanquet method only started in the late 1980s. One of the pioneering works was on the coastal vegetation of Albania (Mullaj 1989), while studies of halophytic and psammophytic vegetation have only recently emerged (Imeri et al. 2010; Fanelli et al. 2015). These differences in the degree of vegetation research along the Adriatic coast is evident from the distribution maps of the localities of sand dunes, on which the Adriatic coast must still be considered main spot in Europe (Géhu 1989; Trinajstić 1989a). The main reason of this research is: (a) to present the current state on the distribution of psammophytic vegetation along the Albania coast, (b) to determine the main vegetation types in real time (c) to describe these vegetation types from a floristic and taxonomic point of view (d) to emphasize the importance of dune coastal vegetation (e) to propose to government authorities the protection statues of these types of habitats (f) to contribute to save these areas from urbanization. About half of the population of the Albania, live in the coastal region. Economic activities in the coastal areas are constantly expanding. Permanently increasing tourism and pollution has already resulted in disruption of or highly negative impacts on fragile ecosystems, impacts on quality of life of resident populations and loss of habitats and species. Present and future trends concerning adverse global phenomena, climate change in particular, are expected to worsen the situation. Coastal area is considered as one of the most important areas in Albania based on the high biodiversity values and the number of habitat. Currently, (1. *Drini Bay Velipoje-Shengjin-Kune-Vain-Patok area, 2. Lalzi Bay, 3. Durresi Bay, 4. Segmenti kala e Turres-derdhja e lumit Shkumbin, 5. Derdhja e lumit Shkumbin-derdhja e lumit Seman Divjake-Karavasta area, 6. Derdhja e lumit Seman-derdhja e lumit Vjose, 7. Vlora Bay-Narta area.)* could be threatened by alteration of their functions which means degradation of their values. In the last years, we have witnessed a growing global awareness concerning habitat monitoring and conservation; habitats as a whole are probably more useful indicators of ecosystem functioning compared to individual species (Balmford et al. 2002; Cowling et al. 2004; Bunce et al. 2013; Gigante et al. 2016a). The protection of biodiversity requires a constant and rigorous technical-scientific commitment at national level, which should also be extended to the obligations deriving from community rules. In particular, the Habitats Directive (92/43/EEC) requires to implement surveillance on the conservation status of habitats and species of Community Interest, taking into account also the most important threat factors that influence their future prospects (Angelini et al. 2016; Gigante et al. 2016b). Monitoring and reporting are of strategic importance to determine the effectiveness of the implementation of the Community Directives on biodiversity. Moreover, they serve as a reference framework to identify priorities and critical issues for the next monitoring period (Genovesi et al. 2014).

METHODS

The study site is composed of beach-dune systems extending alongshore Adriatic Coast Albania.

This ideal natural laboratory (with about 284 km stretching), has been used for assessing beach-dune evolution and community composition. The coastal dune system has been softly managed by marram grass planting and branching, particularly to prevent the development of blowouts. We conducted a detailed bibliographic research on published studies regarding Albania costal dunes vegetation. Highlighting phytosociological relevés regarding plant communities of embryo dunes, mobile dunes, transition dunes, fixed dunes or wooded dunes). For each EU habitat we presented a brief description with the list of diagnostic species based on the European Manuals of the 92/43/EEC Habitats Directive (European Commission, 2007; Biondi et al., 2009). We also included the list of rare and threaten plants and their statues based on the IUCN Codes.

There are nine IUCN Red List categories:

1. Extinct (EX) – No known individuals remaining
2. Extinct in the wild (EW) – Known only to survive in captivity, or as a naturalized population outside its historic range
3. Critically endangered (CR) – Extremely high risk of extinction in the wild
4. Endangered (EN) – High risk of extinction in the wild
5. Vulnerable (VU) – High risk of endangerment in the wild
6. Near threatened (NT) – Likely to become endangered in the near future

7. Least concern (LC) – Lowest risk (does not qualify for a more at-risk category; widespread and abundant taxa are included in this category)
8. Data deficient (DD) – Not enough data to make an assessment of its risk of extinction
9. Not evaluated (NE) – Has not yet been evaluated against the criteria

The species extinction risk assessment is based on the Guidelines for IUCN Categories and Criteria Application version 4.0 (IUCN 2012a, 2012b).

We highlighted those phytosociological associations with their code and particular distribution pattern and finally, for each habitat we suggested major threats.

Fig.1 *Sampling sites (Sites are selected based on the previous experience)*

1. Drini Bay Velipoje Shengjin Kune-Vain-Patok areas,
2. Lalzi Bay,
3. Durresi Bay,
4. Segmenti Kala e Turres-derdhja e Lumit Shkumbin,
5. Derdhja e Lumit Shkumbin-derdhja e Lumit Seman, Divjaka-Karavasta Area.
6. Derdhja e Lumit Seman-derdhja e lumit Vjose.
7. Vlora Bay (Narta area)

These sampling points are the main costal habitats that express the dune vegetation in follow habitates:

- 1210 Annual vegetation of drift lines
- 2110 Embryonic shifting dunes
- 2120 Shifting dunes along the shoreline with Ammophila arenaria (‘white dunes’)
- 2130 * Fixed coastal dunes with herbaceous vegetation (“grey dunes’)
- 2250* Coastal dunes with Juniperus spp. (Juniperus oxycedrus subsp. macrocarpa)
- 2270* Wooded dunes with Pinus pinea and/or Pinus pinaster

All relevés follow the phytosociological method of ZurichMontpellier school (Braun-Blanquet, 1932, 1964). This analysis conducted with the support of Flora of Albania (Paparisto et al.,1984-2000) and Flora Europaea (Tutin et al., 1964-1980). The nomenclature followed is Paparisto et al.,1984-2000. Number, size, distribution pattern of the stations and transects depend on

the size and heterogeneity or diversity of habitats situated in the area of coastal areas, as well as on the bio-ecological characteristics of the species or group of species. The primary focus of this paper was to assess the conservation status of Annex I sand dune habitats in Albania at a representative sample of sand dune sites. The conservation assessment methodology was designed to complement the EU guidelines on assessing conservation status under Article 17 of the Habitats Directive (Evans and Arvela, 2011).

RESULTS

Results demonstrate plant communities on sand dunes along Adriatic that consists of six Habitat types. All releves (*shown on Tab 1*), represent a floristic situation in real time. Unfortunately, some plants of white dunes are disappeared and grey dunes are really in the emergency.

Tab. 1. *Coastal dune habitat and main costal Adriatic regions*.

Habitat types	**Regions**						
	Drini Bay Velipojë, Shëngjin, Kune –Vain, Patok areas	*Lalzi Bay*	*Durrësi Bay*	*Segmenti Kala e Turrës – Derdhja e Lumit Shkumbin*	*Derdhja e Lumit Shkumbin – Derdhja e Lumit Seman (Divjaka-Karavasta Area)*	*Derdhja e Lumit Seman - Derdhja e Lumit Vjosë*	*Vlora Bay – Narta Area*
1210. Annual vegetation of drift line.	++	++	+	++	+++	+++	++
2110 Embryonic'shifting dune	++	++	+	++	++	++	++
2120 Shifting dunes along the shoreline with Ammophila arenaria (white dunes)	+	+	-	++	+	++	++
**2130 Fixed coastal dunes with herbaceous vegetation (grey dunes)*	+	-	-	-	-	-	-
**2250, Dunes with Juniperus oxycedrus ssp. macrocarpa.*	+	-	-	-	++	+	+
**2270 Dunes with Pinus halepensis, pinaster, pinea.*	++	++	++	++	+++	+++	++

(Experiences for more than 20 years with such these habitats, based on the NATURA 200 code and specifics of Mediterranean terrain is established below table by authors).

- + Rare, ++ Abundant, +++ Frequent
- The code corresponds to the NATURA 2000 code.
- The sign'*' indicates priority habitat types.

The floristic inventory includes 220 plant species. Among the species characteristic for the class Cakiletea maritimae most common were *Xanthium orientale ssp. italicum* and *Cakile maritima*,

while among the common species of the Ammophiletea were *Elytrigia juncea, Echinophora spinosa, Eryngium maritimum, Euphorbia paralias* and *Medicago marina.*

There is an evident increase of some psammophytic species towards the south of studied area: *Glaucium flavum, Matthiola tricuspidata, Polygonum maritimum, Ammophila arenaria, Ephedra distachya, Achillea maritima* and *Sporobolus pungens*. Rare psammophytes in the studied area were *Ephedra distachya, Stachys maritima*, and *Ononis variegata*.

During the field trips in the Adriatic region, *(1. Drini Bay Velipoje-Shengjin-Kune-Vain-Patok area, 2. Lalzi Bay, 3. Durresi Bay, 4. Segmenti kala e Turres-derdhja e lumit Shkumbin, 5. Derdhja e lumit Shkumbin-derdhja e lumit Seman Divjake-Karavasta area, 6. Derdhja e lumit Seman-derdhja e lumit Vjose, 7. Vlora Bay-Narta area.).* These plants are studied in floristic and phytosociological point of view. Dominant families are Compositae and Graminae, followed by Leguminosae and Plantaginaceae.

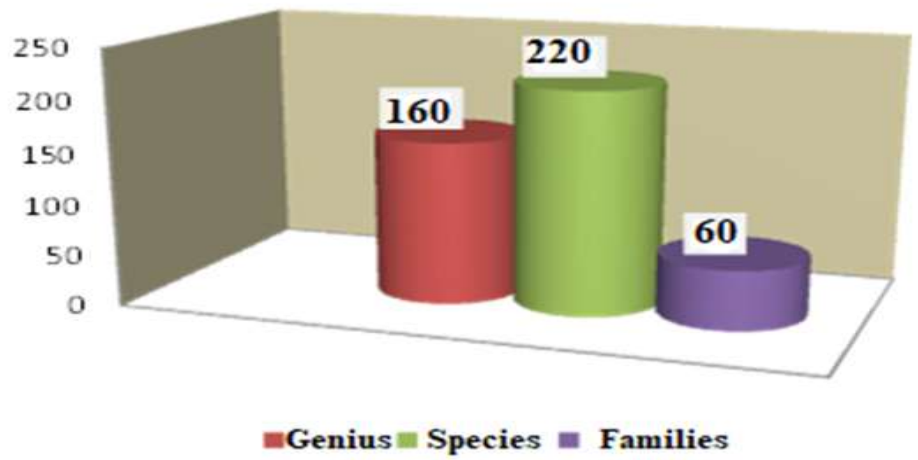


Fig 2. *Dominant families in coastal dunes habitates*.

The plants of sand beaches and dunes consist largely of specialist species. Sand dunes in *Drini Bay*, are very rich in plant species and breeding birds. The “aquatic” vegetation is important for many fish species, both for typical coastal fish and for many fish of the open sea that pass their first life stages in miseries of the coastal zone. Dunes are characterized by very specific plant species, especially dominated by the succulent species. The focus of the study was also the evaluation of rare and threaten plants in these sites in order to give the proper decision for the threaten and endangers plants. **Tab 3** shown the present situation about the threaten statues of the plants in different areas. Their statues according IUCN and the priority objectives for protection of the species. In all areas there are critically endangered species and habitats of global, regional or national importance. Costal habitats are distinguished for their endemic species or biodiversity.

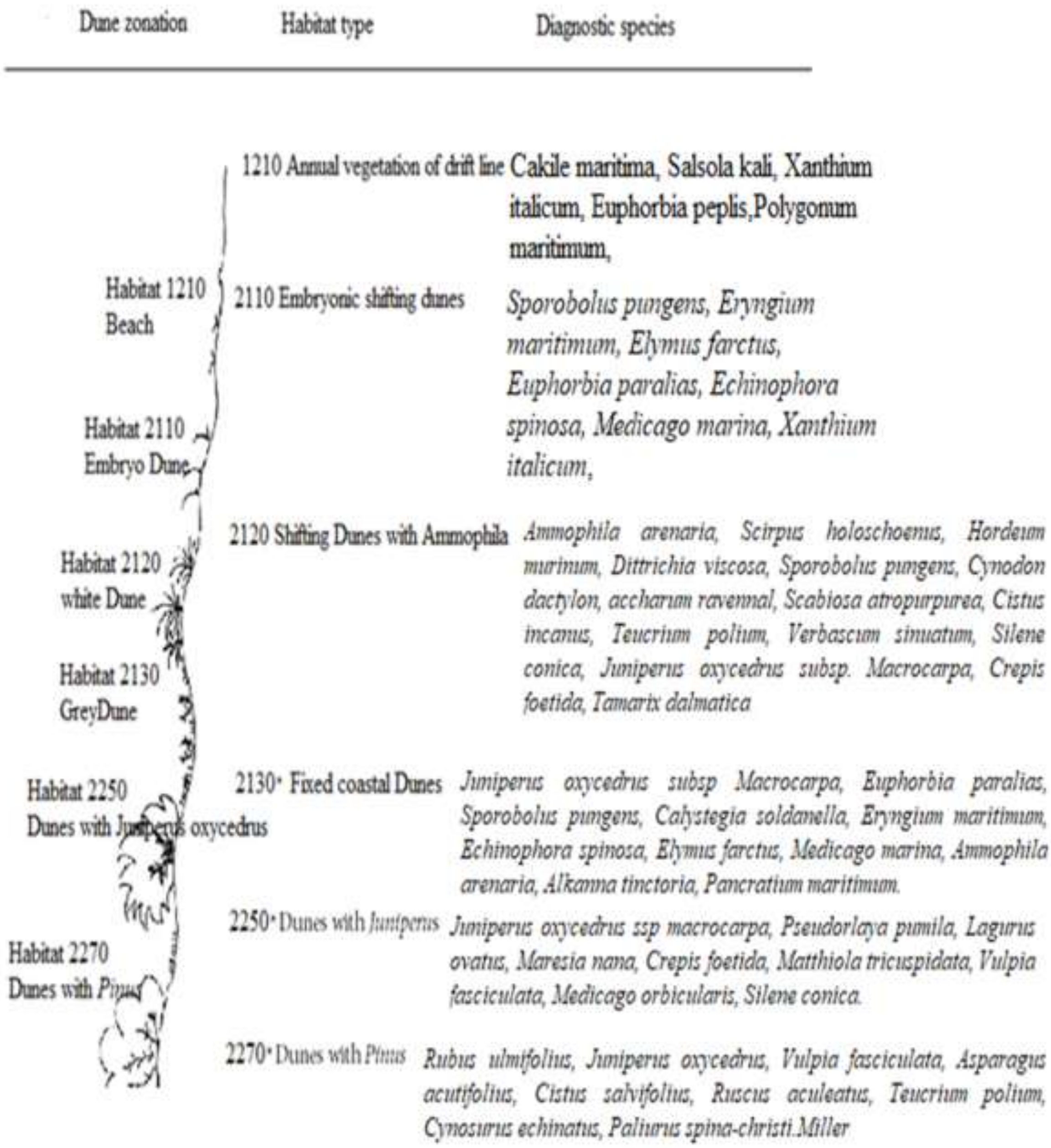


Fig 3. *Scheme of habitat types in Coastal dunes in Adriatic coast line with corresponding diagnostic plant species. (This scheme is worked in Paint program; we have all samples in national herbarium in the Botany chair at Agricultural University of Tirana, Albania.*

In Adriatic coast line there are 6 natural habitates:

1210 Annual vegetation of drift lines

1) Formations of annuals or representatives of annuals and perennials, occupying accumulations of drift material and gravel rich in nitrogenous organic matter (Cakiletea maritimae p.).

2) Plants: *Cakile maritima, Salsola kali, Atriplex spp, Polygonum spp., Euphorbia peplis, Mertensia maritima, Glaucium flavum, Matthiola sinuata, M. tricuspidata, Euphorbia paralias, Eryngium maritimum*.

3) Associations: - Cakilo-Xanthietum strumarii Beguinot 1941, Pign. 1953

2110 Embryonic shifting dunes

1) Formations of the coast representing the first stages of dune construction, constituted by ripples or raised sand surfaces of the upper beach or by a seaward fringe at the foot of the tall dunes.

2) Plants: - *Elymus farctus (Agropyron junceum), Sporobolus pungens, Euphorbia peplis, Medicago marina,Eryngium maritimum, Pancratium maritimum.*

3) Associations: -Cakilo-Xanthietum strumarii, Beguinot 1941, Pign 1953.

2120 Shifting dunes along the shoreline with Ammophila arenaria (white dunes)

1) Mobile dunes forming the seaward cordon or cordons of dune systems of the coasts

2) Plants: - *Ammophila arenaria, Eryngium maritimum, Euphorbia paralias, Calystegia soldanella, Otanthus maritimus, Medicago marina, Cyperus capitatus*.

3.) Associations: - Medicagini marinae-Ammophiletum australis Br.-Bl. 1921 corr. F. Prieto & T.E. Díaz 1991

2130 *Fixed coastal dunes with herbaceous vegetation (grey dunes)

1) Fixed dunes, stabilized and colonized by more or less closed perennial grasslands and abundant carpets of lichens and mosses.

2) Plants: - *Echinophora spinose, Alkanna tinctoria, Elymus farctus, Medicago orbicularis, Calystegia soldanella, Pseudorlaya pumila, Lagurus ovatus, Matthiola tricuspidat*a.

3). Associations: Pistacio lentisci-Juniperetum macrocarpae Caneva, De Marco e Mossa (1981)

2250 * Coastal dunes with Juniperus spp.

1) Juniper formations Mediterranean and thermo-Atlantic coastal dune slacks and slopes Juniperus communis formations of calcareous dunes.

2) Plants: *Juniperus oxycedrus ssp. macrocarpa, Euphorbia paralias L. Sporobolus pungens, Calystegia soldanella, Eryngium maritimum, Echinophora spinose, Elymus farctus, Medicago marina.*

3) Associations: - Juniperetum macrocarpae, Pistacio lentisci-Juniperetum macrocarpae

2270 * Wooded dunes with Pinus pinea and/or Pinus pinaster

1) Coastal dunes colonised by Mediterranean and Atlantic thermophilous pines, corresponding to the substitution facies or in some stations climax formations of evergreen oak of artificial origin Quercetalia ilicis.

2) Plants: *Pinus pinea, P. pinaster, P. halepensis, Juniperus macrocarpa, Phillyrea angustifolia, Rubus ulmifolius, Vulpia fasciculate, Asparagus acutifolius, Cistus salvifolius, Ruscus aculeatus, Teucrium polium, Cynosurus echinatus.*

3). Associations: -Pinea-pinaster.

Tab. 3. *IUCN categories and criteria. The species extinction risk assessment is based on the IUCN Red List Categories version 3.1 (IUCN 2001), on the Guidelines for the use of the IUCN Red List Categories and Criteria version 10 (IUCN 2013a), and on the Guidelines for IUCN Categories and Criteria Application version 4.0 (IUCN 2012a, 2012b). The risk categories are 9 and include:*

No.	Latin Species Name	Name in Albanian language	New speciesname	Existing National Threat Category and Criteria (2013)	Updated National Threat Category and Criteria (2019)	IUCN Current Threat Category and Criteria at Global, European and Mediterranean scale	Main reasons for National threat status being updated/revised
1	*Pinus pinea* L.	Pisha e butë		VU A2c	NT	LC	The main risk comes from woodcutting, but the pressure is not high
2	*Pancratium maritimum* L.	Zambak deti		EN A1b	EN A1b	LC	The threat category remains unchanged
3	*Aster albanicus subsp. paparistoi*	Aster shqiptar	*Galatella albanica* Degen	EN A1b	**VU B2**		According to Kosovo Red Book and in country assessments
4	*Matthiola tricuspidata* (L.) W. T. Aiton	Pllatkë trithimthore		EN A1b	EN A1b		The threat category remains unchanged
5	*Ephedra distachya* L.	Gjunjëz		EN A1b	**VU-AL-A3c**	LC	The species itself is present in sandy dunes of coastal area of Albania, but its habitat is exposed to risk, due to human intervention, which makes the species habitat vulnerable. One location in Durres (Golem beach) is already extinct.
6	*Quercus ilex* L.	Ilqe, lëqeshtë, hilqe, lëqeshtë, ylnjë, ylqer		EN A1b	NT	LC	The species has a wide range of distribution, mostly in the western hills of the country. The specimens are not endangered but still its habitat is under the anthropogenic pressure.
7	*Stachys maritima* Gouan	Sarushë bregdetare		VU A1b	VU A1b		The threat category remains unchanged
8	*Anacamptis morio subsp. caucasica* (K. Koch) H. Kretzschmar, Eccarius & H. Dietr. (=Orchis albanica)	Salepi shqiptar		EN A1b	EN A1b		The threat category remains unchanged
9	*Glaucium flavum* Crantz	Glauku i verdhë		EN A1b	EN A1b	LC	The threat category remains unchanged
10	*Ammophila arenaria* (L.) Link	Amofilë e ranishteve		EN A1b	EN A1b		The threat category remains unchanged

The importance of this area is illustrated by the fact that many of the threatened plant species in the Red Book of Albania occur in this area. In general, the natural ecosystem of Costal Adriatic can be seen as an essential part of Europe's natural heritage.

There are a lot of problems with costal environment and habitat loss is probably the most Important. A number of coastal plant species are now believed to have become extinct due to total destruction of their habitats, especially sand beaches.

Along the Albania, Adriatic coast there is a wide variety of psamophyllus plant community these areas are characterized by high species richness and by endemic and rare associations. Owner investigation was focused in Adriatic coast dunes and the time span of relevés concerns more than 10 years, it is also probable that in some areas the presence of coastal habitats could have been overestimated. In fact, some communities may have disappeared due to a more recent habitat loss. In addition, it is also possible that in other areas habitats were underestimated because of the lack of published phytosociological studies. The results of the meta-analysis showed that community patterns shift from annual beach communities to shrub-covered fixed dunes, probably associated with changes in the environmental characteristics. Dunes are among the most threatened habitats at national level, mainly due to anthropic activities. As it is highly probable that human development and recreational activities along coasts will continue to be intense, an appropriate management of well-preserved dune systems, paired with the restoration of the more damaged ones, are urgently needed. The information derived from this database could be used for the monitoring and surveillance of EU habitats and species, as required by Habitats Directive (EEC, 1992), as well as for the "Natura 2000" ecological network management (Boillotet al., 1997). An adequate implementation of the Habitats Directive will help to guarantee that coastal dunes retain their major features, promoting their conservation for the future generations.

Sintaxonomical review is presented in following list.

1. **CAKILETEA MARITIMAE** Tüxen & Preising ex Br.-Bl.& Tüxen 1952

Euphorbietalia peplis Tx.ex Oberd. 1949

Euphorbion peplis Tx. ex Oberd. 1952

Cakilo - Xanthietum strumarii (Beg. 1941) Pign. 1958

2. **AMMOPHILETEA** Br.-Bl. & Tüxen ex Westhoff, Dijk, Passchier 1946.

Ammophiletalia Br.-Bl. 1933

Agropyrion juncei Pignatti 1953

Eryngio-Sporoboletum virginici Gehu et Uslu 1989

Euphorbio paraliae-Agropyretum junceiformis Tüxen in Br.-Bl. & Tüxen 1952 corr. Darimont, Duvigneaud & Lambinon 1962

Ammophilion australis Br.-Bl. 1921 corr. Rivas-Martínez, Costa & Izco in Rivas-Martínez, Lousã, T.E. Díaz, Fernández-González & J.C. Costa 1990

Medicagini marinae-Ammophiletum australis Br.-Bl. 1921 corr. F. Prieto & T.E. Díaz 1991

3. Kl. QUERCETEA ILICIS Br. Bl. 1947
*Rend. Pistacio lentisci – Rhamnetalia alaterni Rivas – Martinez 1975
+ Al. Juniperion turbinatae Rivas – Martinez 1975 corr. 1987
Asoc. Pistacio lentisci-Juniperetum macrocarpae Caneva, De Marco e Mossa (1981)
+ Al. Quercion ilicis (Br. Bl. 1936) Riv. Martinez 1975
Asoc. Pinetum halepensis – pineae

4. Kl. JUNCETEA MARITIMI Br.-Bl. 1952 em. Beeftink 1965
* Rend. Juncetalia maritimi Br.-Bl. 1931
+ Al. Plantaginion crassifoliae Br.-Bl. in Br.-Bl., Roussine & Nègre 1952
Asoc. Eriantho-Schoenetum nigricantis (Pignatti 1953) Géhu in Géhu et al. 1984
Asoc. Holoschoenetum romani Tchou 1948

The floristic complex of the ***Llazi bay*** area dunes, *begin Eryngium maritimum, Cakile maritima, Echinophora spinosa, Euphorbia paralias* and *Ammophila arenaria*. They form almost the entire vegetation of the beaches and the shifting dunes. Perennial plants as a biological type dominate in floristic complexes, though in some cases (mainly on the beaches) the dominants are pioneer annual plants (*Cakile maritima, Salsola kali or Euphorbia peplis*). Analyzing the transversal profile of a dune (Uslu & Géhu 1990), starting from the shoreline, where the waves break, and continuing towards the inner part of the shore, one can observe a sequence of vegetation clusters which determine various habitats and various stages of growth of the dune's sandbar.
In accordance with the specified definition of psammophytic vegetation succession dynamics and stages of dune formation, the following parts of the dune complexes have been evidenced:
• The higher parts of the beaches with pioneer vegetation
• Embryonic dunes
• Shifting dunes
Pioneer vegetation on the higher parts of the beaches.
This association represents the first stages of development of littoral psammophytic vegetation in the higher beach places. Despite the poor floristic composition, the total abundance of the species is often very low. Pioneer plants are so called because they are the first plants capable of colonizing this type of hostile environment. The hostility is caused by strong thermal changes, poorness of water and finally, because of thevariable saline content. The most representative plants among them are Cakile maritima, Salsola kali, Inula crithmoides and Xanthium strumarium. These dune morphology is clearly expressed in *Drini Bay Velipoje-Shengjin-Kune-Vain-Patok* area, in this area **Embryonic dunes** gradually going away from the coast line and as the height of sandy dunes is increased, the physiognomy of vegetation is imparted by the species *Eryngium maritimum, Euphorbia paralias, Echinophora spinosa, Elymus farctus, Cyperus capitatus, Sporobolus pungens,* that pertain to a more evolved phase of psammophytic vegetation and from the beaches to the embryonic dunes. This type of vegetation represents a stable "potential" of

the sandy banks. The discussed vegetation, in most of the cases, is under human impact determined by the developing tourism, intensive usage and intensive erosion. An obvious result of human impact is the expansive distribution of *Xanthium strumarium subsp. italicum* in the highest beach places and the embryonic dunes. The species was introduced from America and is perfectly adapted in these areas.

Shifting dune vegetation is well expressed in *Segmenti Kala e Turrës – Derdhja e Lumit Shkumbin, Derdhja e Lumit Seman - Derdhja e Lumit Vjosë, Vlora Bay Narta-area,* the architecture of dune height is accompanied as well with the gradual change of the physiognomy of this vegetation. The highest dunes are colonized by the big tufts of Ammophila arenaria which grow especially on the crest of the dunes. This species is the real builder of the dunes. The presence of this species is an important factor in impeding the movement of sand quantities pushed away by the sea winds towards the continent. From this type of vegetation there are noticed two evaluative lines:

Retro dune or degradation of sandy dunes and the formation of depressions. The end of depressions is closer to the level of salted ground waters. The ground becomes wetter and different vegetation grows from that of dunes, dominated by *Erianthus ravennae, Scirpus holoschoenus, Schoenus nigricans* and *Plantago coronopus.*

Mediterranean pine forest: These forests occupy a considerable part of the Lalzi Bay area distributed mainly on sandy dunes. In generally, they represent relatively young forests, cultivated recently in order to stabilize the sandy dunes and protect the agricultural lands. The physiognomy of this formation is imparted by the species *Pinus halepensis* and *Pinus pinea*. The shrubby layer is represented by typical Mediterranean species. The most spread shrubs in this formation are *Myrtus communis, Juniperus oxycedrus subsp. macrocarpa, Erica manipuliflora* and *Pistacia lentiscus*. These forests constitute the last most evolved phase of the vegetation of sandy dunes.

On the other side monitoring along the coastline of Narta area from the delta of Vjosa river up to the Old Beach of Vlora. This region is known for some of the largest dunes of Albania, which altitude varies from 1-2 m to 4-5 m. The sandy belt along the coastline is completely bare of vegetation to a length sometimes extending up to 30 m. The lack of vegetation in this belt is a result of the active life of different animals, mainly crustacean species. After the first belt from the sea shoreline, the Phanerogamy vegetation appears in mudded belt, at the sandy belt area, which is already washed away by the considerable amounts of salt as a result of rainwaters.

Pioneer species, *Cakile maritima, Xanthium strumarium subsp. italicum, Salsola kali,* isolated at the first part of this belt, become more frequent when leaving the coastline. The vegetation of this sandy belt belongs to the pioneer association Cakilo-Xanthietum italici.

Gradually, going away from the coastline and as the altitude of sandy dunes is increasing; the physiognomy of vegetation is composed by species as *Ammophila arenaria subsp arundinaceae, Elymus farctus, Echinophora spinosa* etc. On sand dunes, these species are considered as important dune building plants. *Ammophila arenaria subsp. arundinacea* represents the characteristic specie of the association Ammophiletum. The degradation of sandy dunes and formation of depressions is accompanied by a different vegetation, dominated mainly by *Sporobolus punges*, characteristic specie of the association Sporoboletum. Ammophiletum association constitutes the last most evolved phase of the vegetation of sandy dunes or the borderline between dune vegetation and the Mediterranean pine forests. These forests occupy a considerable part of the Narta area, extending parallel with dune systems of this area. These forests are relatively of a young age, cultivated (30-40 years ago) recently in order to stabilize the sandy dunes and protect the agricultural lands.

The physiognomy of this formation is composed by the species *Pinus maritima, P. pinea, P. pinaster* (tall ligneous trees, the height of 10 year trees is about 5-7 m and the cover about 70-80% of total area). The shrub layer is represented by typical Mediterranean species such as *Pistacia lentiscus, Erica manipuliflora, Myrtus communis* etc, characteristic species of the Class Quercetea ilicis. The pine forests generally originated from plantations, cultivated many years ago for sandy
dune stabilisation and to protect arable land. However, it is now a feature of many
Mediterranean coastlines and currently they represent habitats with priority status, included in
Annex I to Directive 92/43/EEC. The forest is dominated by *Pinus halepensis* and *P. pinea* growing densely on sandy dunes. Generally, these forests are well preserved, with mature trees and very dense canopy cover. As a result of the high shade cast, there is an almost total
absence of shrubby and herbaceous under-storeys under mature trees. In clearings, shrubby and herbaceous understories exist, dominated by species such as Elm leaf blackberry (Rubus
ulmifolius), as well as Common myrtle (*Myrtus communis*), Heath (Erica manipuliflora),
Mastic tree (Pistacia lentiscus), Prickly juniper (Juniperus oxycedrus subsp. macrocarpa),
Rabit-tail (Lagurus ovata), etc. Plants: *Pinus pinea, P. halepensis, Rubus ulmifolius, Myrtus communis, Erica manipuliflora, Pistacia lentiscus, Juniperus oxycedrus subsp. macrocarpa, Lagurus ovata*, etc.

Recomandation and perspective job

1. We should highlight that the key dune habitat 2130 (Fixed coastal dunes with herbaceous vegetation) are threaten and should have a conservation status for all the criteria in both Biogeographical Regions.

2. Moreover, our results showed that both *Fixed coastal dunes with herbaceous vegetation* and *Dunes with Juniperus oxycedrus ssp. macrocarpa* Habitats are highly threatened.

3. The overall worrying state of conservation of the dune systems claims for a better knowledge of pressures and threats acting on these habitats and for further monitoring plans.

4. Without such a systematic approach, coastal dune habitats are going to face further degradation trends in both structure and functions, including also the disruption of spatial zonation of plant communities (Sarmati et al. 2019). This degradation process could finally

lead to a dramatic alteration of the ecosystem services they provide (Everard et al. 2010; Barbier et al. 2011; Drius et al. 2019). Additionally, degraded dune habitats are more susceptible to biological invasions (Del Vecchio et al. 2015; Gheza et al. 2018; Giulio et al. 2020).

5. In dynamic and vulnerable ecosystems such as coastal dunes, successful conservation outcomes in the long-term depend on sound evaluations of the effectiveness of current management measures, supported by regular and highly frequent on-ground monitoring, both inside and outside protected sites. We believe that our results provide bases and useful insights for dune habitats protection and management, in the context of the monitoring and reporting obligations set up by the Habitats Directive.

6. Therefore, we should clarify that for dune Habitats, some of the changes in distribution and trends might probably be related to the application of more accurate and updated data. However, even though the geographical distribution maps of these Habitats have been validated by long term experience in coastal field trips. Finally, it's worth noting that the information collected has been structured to ensure the archiving and traceability of both published and unpublished literature, placing a solid base of reliable and verified data at the local scale for the next reporting cycles (Gigante et al. 2019).

REFERENCES

Boillot, F, Vignault M P, De Benito M J, 1997, Process for assessing national lists of proposed Sites of Community Interest (pSCI) at biogeographical level. Nature und Landschaft 72, 474-476.

Braun-Blanquet J. 1932.Ammophiletalia et Salicornietalia méditerranéens. 23 p.

Braun-Blanquet J. 1964. Pfl anzensoziologie. Grundzüge der Vegetationskunde. Springer Verlag, Wien–New York, 865 pp

Chytrý M, Tichý L, Kolt J & Botta- Dukát Z. 2002. Determination of diagnostic species with statistical fidelity measures. Journal of Vegetati on Science 13: 79–90.

Paparisto K, Qosja Xh, Demiri M, Mitrushi J, Balza E, Ruci B & Mullaj A. 1984-2000. Flora e

Shqipërisë. (Vol.1- 4) Akademia e Shkencave, Tiranë

Pignatti S. 2005. Valori di bioindicazione delle pi ante vascolari della fl ora d'Italia (Bioindicator values of vascular plants of the Flora of Italy). Braun–Blanquetia 39: 1–96.

Tutin TG, Heywood VH, Burges NA, Valentine DH, Walters SM & Webb DA. 1964-1980. Flora Europaea: Volume I – V. Cambridge University Press. Cambridge.

Uslu T. & Géhu JM. 1990. Syntaxonomic units and flora of Turkish coastal dunes. Proceedings of the 2 nd Int. Colloquy on the Mediterranean Coasts and the Protection of the Environment, Council of Europe, Strasbourg 42 pp.

Alegro, A., Biljaković, M., Bogdanović, S. & Boršić, I. 2003. Sporobolus pungens (Schreber) Kunth (Poaceae), rare and endangered psammophytic species in Croatia. Natura Croatica 12: 1–7.

Alegro, A., Biljaković, M., Bogdanović, S. & Boršić, I. 2004. Psammo-halophytic vegetation on the largest sand area on the Croatian coast: the island of Mljet, southern Adriatic. Biologia 59: 435–445.

Attorre, F., Maggini, A., Di Traglia, M., De Sanctis, M. & Vitale, M. 2013. A methodological approach for assessing the effects of disturbance factors on the conservation status of Mediterranean coastal dune systems. Applied Vegetation Science 16: 333–342.

Beck von Mannagetta, G. 1901. Die Vegetationsverhältnisse der Illyrischen Länder. In: Engler, A. & Drude, O. (eds.) Die Vegetation der Erde. Sammlung pflanzengeographischer

Monographien. 536 pp. + map. Verlag W. Engelmann, Leipzig, DE. Biondi, E. 1989. The vegetation of sedimentary low coasts in Corfu island. Colloques phytosociologiques 19: 401 427.

Biondi, E. 2007. Thoughts on the ecology and syntaxonomy of some vegetation typologies of the Mediterranean coast. Fitosociologia 44: 3–10.

Brullo, S. Giusso Del Galdo G.P., Siracusa G. & Spampinato G. 2001. Considerazioni fitogeografiche sulla vegetazione psammofila dei litorali italiani. Biogeographia 22: 93–137.

Buffa, G., Fantinato, E. & Pizzo, L. 2012. Effects of disturbance on sandy coastal ecosystems of N-Adriatic coasts (Italy). In: Lameed, G.A. (eds.) Biodiversity enrichment in a diverse world, pp. 338–372. InTech, Rijeka, HR.

Carboni, M., Carranza, M.L. & Acosta, A. 2009. Assessing conservation status on coastal dunes: A multiscale approach. Landscape and Urban Planning 91: 17–25.

Chytrý, M., Tichý, L., Holt, J. & Botta-Dukát, Z. 2002. Determination of diagnostic species with statistical fidelity measures. Journal of Vegetation Science 13: 79–90.

Corbetta, F., Gratani, L., Moriconi, M. & Pirone, G. 1989. Lineamenti vegetazionali e caratterizzazione ecologica delle spiagge dell'arco da Taranto alla foce del Sinni [Linear vegetation and ecological characterization of the beaches from Taranto bay to the the mouth of the Sinni river]. Colloques phytosociologiques 19: 461–521.

Doing, H. 1985. Coastal fore-dune zonation and succession in various parts of the world. Vegetatio 61: 65–75.

Fanelli, G., De Sanctis, M., Gjeta, E., Mullaj, A. & Attore, F. 2015. The vegetation of the Buna river protected landscape (Albania). Hacquetia 14: 129–174.

Feola, S., Carranza, M.L., Schaminée, J.H.J., Janssen, J.A.M. & Acosta, A.T.R. 2011. EU habitats of interest: an insight into Atlantic and Mediterranean beach and foredunes. Biodiversity and Conservation 20: 1457–1468.

Géhu, J.M. 1989. Sur la fragilité structurale de la frange terrestre des bords de mer et son analyse par les méthodes de la phytocoenotique moderne dans un souci conservatoire. Colloques phytosociologiques 19: 37–44.

Géhu, J.M. & Biondi, E. 1996. Synoptique des associations végétales du littoral adriatique italien. Plant Biosystems 130: 257–270.

Géhu, J.M., Costa, M., Scoppola, A., Biondi, E., Marchiori, S., Peris, G.B., Franck, J., Caniglia, G. & Veri, L. 1984. Essai systématique et synchorologique sur les végétations littorales italiennes dans un but conservatoire. 1. Dune et vases salées. Documents Phytosociologiques 8: 393–474.

Géhu, J.M., Uslu, T. & Costa, M. 1989. Apport à la connaissance phytosociologique du littoral sud de la Turquie Méditerranéenne. Colloques phytosociologiques 19: 591–622.

Heslenfeld, P., Jungerius, P.D. & Klijn, J.A. 2004. European coastal dunes: ecological values, threats, opportunities and policy development. In: Martínez, M.L. & Psuty, N.P. (eds.) Coastal dunes. Ecology and conservation, pp. 335–351.

Springer, Berlin Heidelberg, DE. Horvat, I., Glavač, V. & Ellenberg, H. 1974. Vegetation Südosteuropas. Gustav Fischer Verlag, Stuttgart, DE. Horvatić, S. 1934. Flora i vegetacija otoka Paga [Flora and vegetation of island Pag]. Prir. Istraž. Jugosl. Akad. 19: 116–372.

Horvatić, S. 1939. Pregled vegetacije otoka Raba s gledištabiljne sociologije [Vegetation survey of the island of Rab from the plant sociological point of view]. Prir. Istraž. Jugosl. Akad. 22: 1–96.

Horvatić, S. 1963. Vegetacijska karta otoka Paga s općim pregledom vegetacijskih jedinica Hrvatskog primorja [Vegetation map of the island of Pag with a general overview of vegetation units of the Croatian coast]. Prir. Istraž. Jugosl. Akad. 33: 1–187.

Imeri, A., Mullaj, A., Dodona, E. & Kupe, L. 2010. Costal vegetation of the Lalzi bay (Albania). Botanica serbica 34: 99–105.

Juračić, M., Benac, Č., Pikelj, K. & Ilić, S. 2009. Comparison of the vulnerability of limestone (karst) and siliciclastic coasts (example from the Kvarner area, NE Adriatic, Croatia). Geomorphology 107: 90–99.

Korica, B. & Lovrić, A.Ž. 1979. Ekologija i zaštita obalnih psamofita sjeveroistočnog Jadrana [Ecology and conservation of coastal psammophytes of NE Adriatic]. In: Rauš, Đ. (eds.) Drugi kongres ekologa Jugoslavije, pp. 205–217. Savez društava ekologa Jugoslavije, Zadar-Plitvice, HR.

Lavrentiades, G. J. 1964. The ammophilous vegetation of the Western Peloponnesos coasts. Vegetatio 12: 223–287.

Maarel, E. van der 1979. Transformation of cover-abundance values in phytosociology and its effects on community similarity. Vegetatio 39: 97–114.

Maarel, E. van der 2003. Some remarks on the functions of European coastal ecosystems. Phytocoenologia 33: 187–202.

Maun, M.A. 2009. The biology of coastal sand dunes. Oxford University Press, New York, US.

McCune, B. & Grace, J.B. 2002. Analysis of ecological communities. MjM Software Design, Glenden Beach, US.

Mijović, A., Popović, Z., Karadžić, B., Mijatović, M. & Perišić, S. 2006. Distribution of xerohalophytic vegetation along the seaward and landward zone in South-Adriatic sandy bach (Montenegro). Biotechnology & Biotechnological Equipment 20: 30–35.

Mijović, A., Popović, Z., Vukov, T., Smiljanić, M., Matić, R. & Bojović, S. 2012. Sand topography influences the distribution of xerohalophytic vegetation on a southern Adriatic beach in Montenegro. Plant Biosystems 146: 664–673.

Miller, T.E., Gornish, E.S. & Buckley, H.L. 2010. Climate and coastal dune vegetation: disturbance, recovery, and succession. Plant Ecology 206: 97–104.

Morton, F. 1915. Pflanzengeograpische Monographie der Inselgruppe Arbe, umfassend die Inseln Arbe, Dolin, S. Gregorio, Goli und Pervicchio samt dem umliegenden Scoglien. Beiblatt zu den Botanischen Jahrbüchern 116: 67–273.

Mucina, L. 1997. Conspectus of classes of European vegetation. Folia Geobotanica et Phytotaxonomica 32: 117–172.

Mucina, L., Bültman, H., Dierssen, K., Theurillat, J.-P., Dengler, J., Čarni, A., Šumberová, K., Raus, T., Di Pietro & Tichý, L. 2016. Vegetation of Europe: Hierarchical floristic classification system of plant, lichen, and algal communities. Applied Vegetation Science 19 (Suppl. 1): 3–264.

Mullaj, A. 1989. Vegjetacioni bregdetar i Shqipërisë [Coastal vegetation of Albania]. Ph.D. thesis, Albanian Academy of Sciences, Tirana, AL.

Nikolić, T. & Topić, J. 2005. Red book of vascular flora of the Republic of Croatia. Categories EX, RE, CR, EN and VU. Ministry of Culture, State Institute for Nature Protection,Republic of Croatia, Zagreb, HR.

Pignatti, S. 2005. Valori di bioindicazione delle piante vascolari della flora d`Italia [Bioindicator values of vascular plants of the Flora of Italy]. Braun-Blanquetia 39: 1–97.

Pignatti, S., Oberdorfer, E., Schaminée, J. H. J. & Westhoff, V. 1995. On the concept of vegetation class in phytosociology. Journal of Vegetation Science 6: 142–152.

Pirone, G. 2014. Notes on the vegetation diversity on the Adriatic and Ionian Italian coasts: the dunes and cliffs. Plant Sociology 51: 7–18.

Simeoni, U., Pano, N. & Ciavola, P. 1997. The coastline of Albania: morphology, evolution and coastal management issues. Bulletin de l'Institut oceanographique n° special 18: 151–168.

Stanisci, A., Acosta, A., Ercole, S. & Blasi, C. 2004. Plant communities on coastal dunes in Lazio (Italy). Annali di Botanica 4: 115–128.

Sýkora, K.V., Babalonas, D. & Papastergiadou, E.S. 2003. Strandline and sand-dune vegetation of coasts of Greece and of some other Aegean countries. Phytocoenologia 33: 409–446.

Trinajstić, I. 1989a. Vegetation of the class Ammophiletea Br.- Bl. et R. Tx. 1943 in the eastern Adriatic littoral of Yugoslavia. Colloques phytosociologiques 19: 387–394.

Trinajstić, I. 1989b. Prilog poznavanju vegetacije priobalnih pješčanih sprudova razreda Ammophiletea u crnogorskom primorju [Contribution to the knowledge of the coastal sandy beaches vegetation of the class Ammophiletea along the coast of Montenegro]. Crnogorska Akdemija Nauka I umjetnosti, Glasnik Odjeljenja Prirodnih nauka 7: 45–51. [In Croatian.]

Trinajstić, I. 1995. Vegetacijske značajke otoka Mljeta [Vegetation characteristics of island Mljet]. Proc. sympos. Prirodne značajke i društevna valorizacija otoka Mljeta, Ekološke monografije 6: 247–269. [In Croatian.]

Trinajstić, I. 2008. Biljne zajednice Republike Hrvatske [Plant communities of Republic of Croatia]. Akademija šumarskih znanosti, Zagreb, HR. [In Croatian.]

Trinajstić, I. & Jasprica, N. 1998. Vegetation of the class Ammophiletea Br.-Bl. et R. Tx. in the southern part of the Croatian littoral (Dalmatia). Natura Croatica 7: 79–83.

Tutin, T.G. et al. (eds.) 1964–1980, 1993. Flora europaea. Vols 1–5 & Vol. 1 2nd ed. Cambridge, Cambridge university Press, UK.

Willner, W. 2006. The association concept revisited. Phytocoenologia 36: 67–76.

THE NATURAL HORMONE OF THE HUMAN BODY, IRISIN, INDUCES APOPTOSIS IN HELA CERVİCAL CANCER CELLS BY SUPPRESSING GLUCOSE METABOLISM

Deniz ŞUMNULU[1]

[1] *Trakya University, Technology Research Development Application and Research Center, Edirne, Turkey*
Corresponding author e-mail: denizsumnulu@trakya.edu.tr

ABSTRACT

Irisin is a natural body hormone that can also be produced synthetically. Although the first research on irisin was on energy metabolism, recently its proliferative and anti-proliferative effects have been investigated in various types of cancer, depending on the type of cancer. In this study, the 48-h proliferation suppressive dose of irisin on the HeLa cervical cancer cell line was determined to be 50 nM. Additionally, when the same dose was applied to mouse healthy liver cell line AML12, 105.37 ± 5.87% viability was detected compared to the control group. As a result of qRT-PCR analyses performed upon application of this dose of irisin to the HeLa cell line, significant decreases in *PI3K*, *Akt*, *mTOR*, *HIF-1α*, *Mn-SOD*, *CAT*, and *GSR* gene expression levels were detected, whereas significant increases were detected in *GLUT1* and *p53* gene expression levels. As a result of flow cytometry analyses performed based on the same application, 71.84 ± 2.49% of ROS-affected and 51.23 ± 3.14% of total apoptotic and necrotic cell amounts were detected in the HeLa cell line compared to the control group. At the same time, depending on this application, apoptotic symptoms in the nuclear structures of HeLa cells were demonstrated by fluorescence microscope imaging. These data have shown that irisin can play an effective role in the fight against many types of cervical cancer, especially HeLa.

Keywords: Apoptosis, *GLUT1*, HeLa, *HIF-1α*, Irisin, ROS

INTRODUCTION

Irisin is a novel adipomyokine peptide that is secreted from skeletal muscle, and it was discovered in 2012 (Bostrom et al., 2012). Irisin is derived from its precursor, fibronectin type III domain-containing 5 (FNDC5), a transmembrane protein that is cleaved by specific proteases (Bostrom et al., 2012; Huh et al., 2012; Timmons et al., 2012). Although the discovery of irisin is still quite recent, initial studies have indicated that irisin, which is abundantly expressed in skeletal muscle, regulates fat metabolism by converting white adipose tissue into brown adipose tissue. (Robets et al., 2013; Hofmann et al., 2014). Building on the research on fat metabolism, subsequent studies have also investigated the benefits of irisin on glucose metabolism (Wang and Pan, 2016; Zhang et al., 2016). In addition to these data, the anti-cancer molecular mechanisms of irisin on various types of cancer have also been investigated.

As is well known, almost all types of cancer cells require extremely high amounts of energy compared to healthy cells in order to perform activities such as unlimited growth, angiogenesis, or inhibition of apoptosis. Cancer cells meet their increased energy needs by converting glucose to lactate in an oxygen-free environment (Gasinska et al., 2013). Sometimes, even when there is a sufficient amount of oxygen in the environment, cancer cells still prefer an anaerobic method by converting glucose to lactate. This phenomenon is called the Warburg effect (Liberti and Locasale, 2016). Generally, this situation occurs when cancer cells are under intense oxidative stress, and under normal conditions, glucose required for anabolic processes such as cancer cell proliferation is provided in this way. The needed glucose is provided by the glucose transporter family known as GLUT. The overexpression of membrane glucose transporters, including GLUT1, has been observed in many malignancies such as breast, colorectal, salivary gland, and stomach cancers (Józwiak and Lipinska, 2012). Despite the high amount of reactive oxygen species (ROS) in cancer cells, the overexpression of the GLUT1 gene to meet the high glucose demand can disrupt cellular homeostasis. This situation can lead to the suppression of cell proliferation and apoptosis. However, to suppress the accumulated ROS and excessive GLUT1 expression and restore homeostasis, the hypoxia-induced factor 1 alpha (HIF-1α) gene is effectively activated in cancer cells (Masson et al., 2012; Köhl et al., 2006). However, as a result of the suppression of glucose metabolism, the necessary energy must be compensated in some way, and this requirement is met by Phosphatidylinositol 3-kinase (PI3K), which supplies the needed ATP (Zhang et al., 2020). Epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) activate PI3K. Activated PI3K then activates protein kinase B (Akt) in the cell membrane (King et al., 2015). Additionally, the mammalian target of rapamycin (mTOR) is activated by PI3K/Akt and subsequently increases the expression of HIF-1α. Increased HIF-1α expression induces VEGF expression, promoting vascular expansion (Park et al., 2014). The increase in VEGF expression leads to the opening of new glucose pathways. Thus, with the facilitated glucose flow, ROS is suppressed, and the increased Akt/mTOR/HIF-1α pathway expression returns to its previous level, restoring homeostasis (Lin et al., 2021).

According to a study, irisin activates the PI3K/Akt pathway in liver cancer (HepG2, SMMC7721) cell lines, particularly at a dose of 2.5 nM, increasing cell viability, migration, and invasion, and reducing the cytotoxic effect of doxorubicin (Dox) (Shi et al., 2017). Another study reported that irisin has no proliferative and adhesive effects on tumor development in endometrial (KLE, RL95-2), colon (HT29, MCA38), thyroid (SW579, BHP7), and esophageal (OE13, OE33) cancer types (Moon and Mantzoros, 2014). In contrast, irisin has been reported to reduce cell count and migration, suppress Nf-κβ expression levels, and promote apoptosis in breast (MCF-7, MDA-MB-231) cancers (Gannon et al., 2015). Additionally, irisin has shown antiproliferative effects in prostate (LNCaP, DU-145, and PC3) cancer cell lines, especially reducing the lifespan of the LNCaP cell line (Tekin et al., 2015). Furthermore, doses of 20-50 nM of irisin have been shown to inhibit proliferation, growth, migration, and invasion in A549 (NSCLC) and NCI-

H446 (SCLC) lung cancer cell lines (Shao et al., 2017), osteosarcoma (U2OS, MG-63) cell lines (Kong et al., 2017), and pancreatic cancer (MIA PaCa-2, Panc03.27) cell lines (Liu et al., 2018).

Despite the proliferative/anti-proliferative effects of irisin on these mentioned cancer cell lines, its effects on cervical cancer cells are not frequently found in the literature. According to World Health Organization data, cervical cancer is the fourth most common cancer in women globally, with approximately 660,000 new cases and around 350,000 deaths in 2022. Cervical cancer is the most well-documented cancer linked to a sexually transmitted infection with human papillomavirus (HPV) (Burd, 2003). HeLa is one of the oldest defined (1951) cell lines related to HPV-associated cervical cancer (Jones, 1997).

Due to the role of irisin in glucose metabolism (Wang and Pan, 2016; Zhang et al., 2016) and the importance of the PI3K/Akt/mTOR/HIF-1α pathway-GLUT1 balance for cell survival in almost all cancer types, including the HeLa cell line (Arsham et al., 2002), this study investigated the proliferative/apoptotic effects of irisin on the HeLa cell line.

MATERIAL AND METHOD

Healthy mouse (AML12, ATCC® CRL-2254) cell line and human cervical cancer (HeLa, ATCC® CCL-2) cell line were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were seeded into 75 cm² flasks (NEST, China) with 10 mL of medium and incubated at 37°C with 5% CO_2 in a Hera Cell CO_2 incubator. A culture medium consisting of DMEM/EMEM/Ham's F-12 (1:1:1 ratio) (Wiesent Multicell, Canada), 5% fetal bovine serum (FBS, Capricorn, Germany), and 1% L-Glutamine (Thermo-Fisher, USA) was used for cell passaging. To protect the cells from bacterial contamination, the medium was supplemented with 100 IU/mL penicillin-streptomycin (Pen-Strep; Thermo-Fisher) antibiotic and 100× antibiotic-antimycotic (Gibco, USA). Cell passaging was carried out approximately every 48 hours, ensuring the cells reached 97% confluency before passaging.

MTT assays were conducted to assess the effective cytotoxic dose of Irisin on AML12 and HeLa cell lines. Cells were plated separately into transparent 96-well plates with a volume of 180 µL per well and a density of 5×10^3 cells per well.1.56 nM, 3.12 nM, 6.25 nM, 12.5 nM, 25 nM, 50 nM, 100 nM, 200 nM of irisin (Cayman Chemical, 11451) applied to both cell lines for 48-h. Cells without treatment served as controls for comparison with the treated groups. At the end of the irisin application period, 20 µL of MTT solution (Biomatik; A3338-5GM, Canada) was added to each well and incubated for 3 hours. After 3 hours, the liquid from each well was removed, and 100 µL of dimethyl sulfoxide (DMSO) was added. Cell viability was determined by measuring the absorbance at 570 nm using a spectrophotometer (Thermo Scientific Multiskan GO, USA). Untreated control cells with irisin were considered 100% viable. Changes in cell viability due to the application of irisin in doses ranging from 1.56 nM to 200 nM on AML12 and HeLa cells were calculated using the formula below:

Cell viability (%) = (absorbance value of the releated dose of the irisin applied to the wells / absorbance value of the control wells) × 100.

HeLa cell line was seeded into 25 cm² culture flasks at a density of 1×10^5 cells per flask, with 3 replicates for irisin treatment and 3 replicates as controls. After the treatment period, total RNA was isolated from each flask using the Column Pure RNA Miniprep kit (ABM Good, Canada) according to the manufacturer's protocol. RNA quantities from each flask were equalized, and cDNA synthesis was performed using the OneScript Plus cDNA Synthesis kit (ABM Good) according to the manufacturer's instructions. The cDNA samples were stored at -20°C until further RT-PCR analysis.

Expression levels of *PI3K*, *Akt*, *mTOR*, *VEGF*, *HIF-1α*, *Mn-SOD*, *GSR*, *CAT*, *GLUT1*, *p53* genes were analyzed in HeLa cell line for irisin treatment, compared to the control groups. Quantification was performed using a qRT-PCR device (QuantStudio 6 Flex, Applied Biosystems). Changes in gene expression were calculated using the $2^{-\Delta\Delta CT}$ formula and normalized to the internal control glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Gene expression levels were analyzed using SPSS Paired Samples T Test (IBM SPSS Statistics 22). Results are presented as the mean and standard error of the mean (mean _ SD). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The PCR conditions and primer sequences used are provided in the Table 1.

Table 1. Primers base sequences and RT-PCR conditions.

Gene Codes	Primer Base Sequences	Real Time PCR Conditions
Gapdh	5'-CAATGCCTCCTGCACCACCA-3' 5'-GATGTTCTGGAGAGCCCCGC-3'	Hold Stage: 1 Cycle 50°C 2 minute 95°C 10 minute PCR Stage: 40 Cycle 95°C 15 second 60°C 1 minute Melt Curve Stage: 1 Cycle 95°C 15 second 60°C 1 minute 95°C 15 second
PI3K	F: 5'-GGTTGTCTGTCAATCGGTGACTGT-3' R: 5'-GAACTGCAGTGCACCTTTCAAGC-3'	
Akt	5'-TTCTGCAGCTATGCGCAATGTG-3' 5'-TGGCCAGCATACCATAGTGAGGTT-3'	
mTOR	F: 5'-AGTGGACCAGTGGAAACAGG-3' R: 5'-TTCAGCGATGTCTTGTGAGG-3'	
VEGF	5'-AGGAGGGCAGAATCATCACG-3' 5'-CAAGGCCCACAGGGATTTTCT-3'	
CAT	F: 5'-TACGAGCAGGCCAAGAAGTT-3' R: 5'-ACCTTGTACGGGCAGTTCAC-3'	
Mn-SOD	5'-TCTGAAGAAGGCCATCGAGT-3' 5'-GCAGATAGTAGGCGTGCTCC-3'	
GSR	F: 5'-AGTCACAAGCTGGGTGGCACTTGC-3' R: 5'-ACTCCTCCACGCCAGAACCCTC-3'	
HIF-1α	F: 5'-CATAAAGTCTGCAACATGGAAGGT-3' R: 5'-ATTTGATGGGTGAGGAATGGGTT-3'	
GLUT1	F: 5'-GATTGGCTCCTTCTCTGTGG-3' R: 5'-TCAAAGGACTTGCCCAGTTT-3'	
p53	F: 5'-CACGAGCGCTGCTCAGATAGC-3' R: 5'-ACAGGCACAAACACACGCACAAA-3'	

Six-well plates were seeded with 1×10^5 HeLa cells, with three replicates for the control and three replicates for the irisin treatment. Following irisin treatment, the Reactive Oxygen Species (ROS) Fluorometric Assay Kit (Elabscience, E-BC-K138-F, USA) was used to measure and compare the levels of ROS in HeLa cells to control cells. The manufacturer's instructions were followed, and flow cytometry (Applied Biosystems, Attune acoustic focusing cytometer, USA) was employed. Furthermore, the apoptotic cell population was assessed utilizing the Annexin V-FITC/PI Apoptosis Kit (Elabscience, E-CK-A211) in accordance with the flow cytometer's manufacturer's recommendations. Changes in ROS-positive and apoptotic cell numbers in HeLa cells, relative to control groups, following irisin treatment were analyzed using SPSS Paired Samples T Test (IBM SPSS Statistics 22). Results are presented as the mean and standard error of the mean (mean _ SD). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

HeLa cells were seeded into 6-well transparent plates (NEST) as a 1×10^5 cells per well. Hoechst 34580 dye (Cayman Chemical, USA) was used for screening alterations in nuclear structures brought on by apoptosis. 1×10^5 cells per well of six-well plates were used to seed the treatment and control cell groups. All of the wells' media was taken out after the treatment time. The cells were then covered with 500 µL of a solution that included 25 mg of Hoechst 34580 dissolved in 10 mL of 1× PBS in each well. For twenty minutes, the plate was incubated at room temperature in the dark. At the conclusion of the incubation time, all liquid was removed from the wells and the cells were twice rinsed with 1× PBS to prevent false positive findings. Images were taken by a fluorescence microscope with a DAPI filter under a 20× objective.

RESULTS AND DISCUSSION

In this study, the possible cytotoxic effects of irisin hormone, which is a natural hormone in both human and mouse bodies and has 100% homology (Bostrom et al., 2012), on AML12, a mouse healthy liver cell line, and HeLa, a human cervical cancer cell line, were investigated. According to the MTT analysis, it was determined that when 1.56 nM-100 nM irisin was applied to the HeLa cell line for 48-h, there was a regular decrease in the amount of cell viability as the irisin concentration increased. On the other hand, compared to the control group in the AML12 cell line, 99.57 ± 2.03% cell viability was detected due to the application of 3.12 nM irisin, whereas an increase in cell viability was detected compared to the control group for all other application doses of irisin (Figure 1).

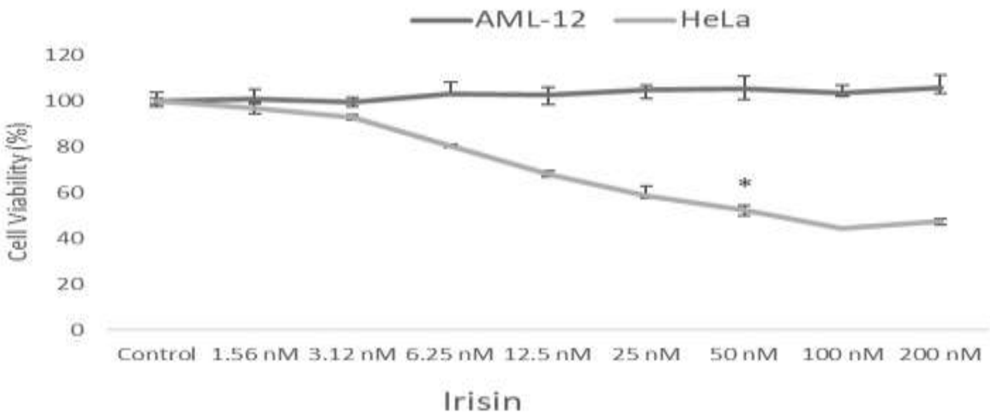


Figure 1. Changes in cell viability due to the application of 3 repeats of irisin to AML12 and HeLa cell lines at a dose range of 1.56 nM - 200 nM for 48-h.

Since 52.21 ± 5.87% viability was detected in the HeLa cell line due to the application of 50 nM irisin and no significant cytotoxic effect was observed for all doses of irisin in the AML12 cell line, it was decided to apply 50 nM irisin to the HeLa cell line for 48-h in the next steps of the study.

It was observed that the expression level of *PI3K* was 0.44 ± 0.03-fold, the expression level of *Akt* was 0.49 ± 0.1-fold, the expression level of *mTOR* was 0.26 ± 0.11-fold, the expression level of *VEGF* was 0.38 ± 0.19-fold, the expression level of *HIF-1α* was 0.12 ± 0.01-fold, the expression level of *Mn-SOD* was 0.23 ± 0.14-fold, the expression level of *GSR* was 0.21 ± 0.03-fold, the expression level of *CAT* was 0.43 ± 0.18-fold, the expression level of *GLUT1* was 6.72 ± 2.25-fold, and the expression level of *p53* was 4.89 ± 1.67-fold changed compared to the control group upon the application of 50 nM irisin to the HeLa cell line (Figure 2).

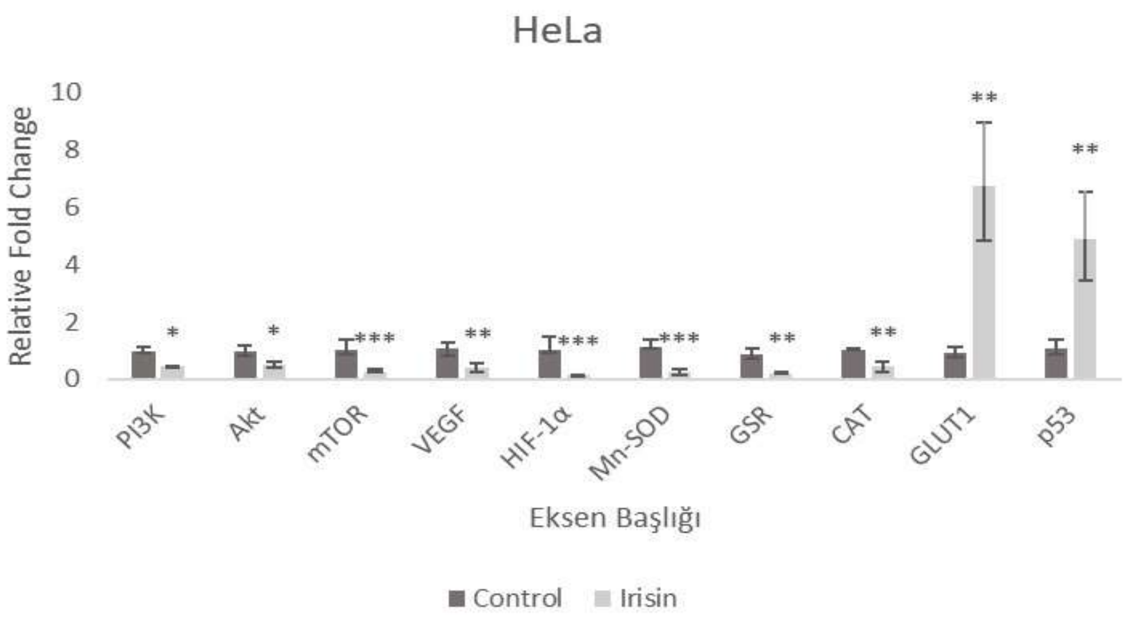


Figure 2. Changes in the expression levels of cell division, antioxidant, hypoxia, and apoptotic genes compared to the control group upon the application of 50 nM irisin for 48-h to the HeLa cell line (Mean= ±SD, *p<0.05, **p<0.01, ***p<0.005).

As seen in Figure 2, despite significant decreases in the expression levels of the PI3K/Akt/mTOR/HIF-1α hypoxia regulatory pathway genes (Lin et al., 2021) due to the application of irisin compared to the control group, the significant increase in GLUT1 expression levels indicates that the energy provided by glucose metabolism is insufficient for HeLa cells. This condition (hypoxia) results in ROS, which cannot be balanced by the antioxidant genes Mn-SOD, GSR, and CAT, ultimately leading to significant increases in the expression levels of the p53 tumor suppressor gene and causing cells to undergo apoptosis (Masson et al., 2012; Köhl et al., 2006).

Additionally, according to the flow cytometry analyses, 71.84 ± 2.49% of cells were affected by ROS and 51.23 ± 3.14% of the total apoptotic and necrotic cells were detected due to the application of irisin (Figure 3).

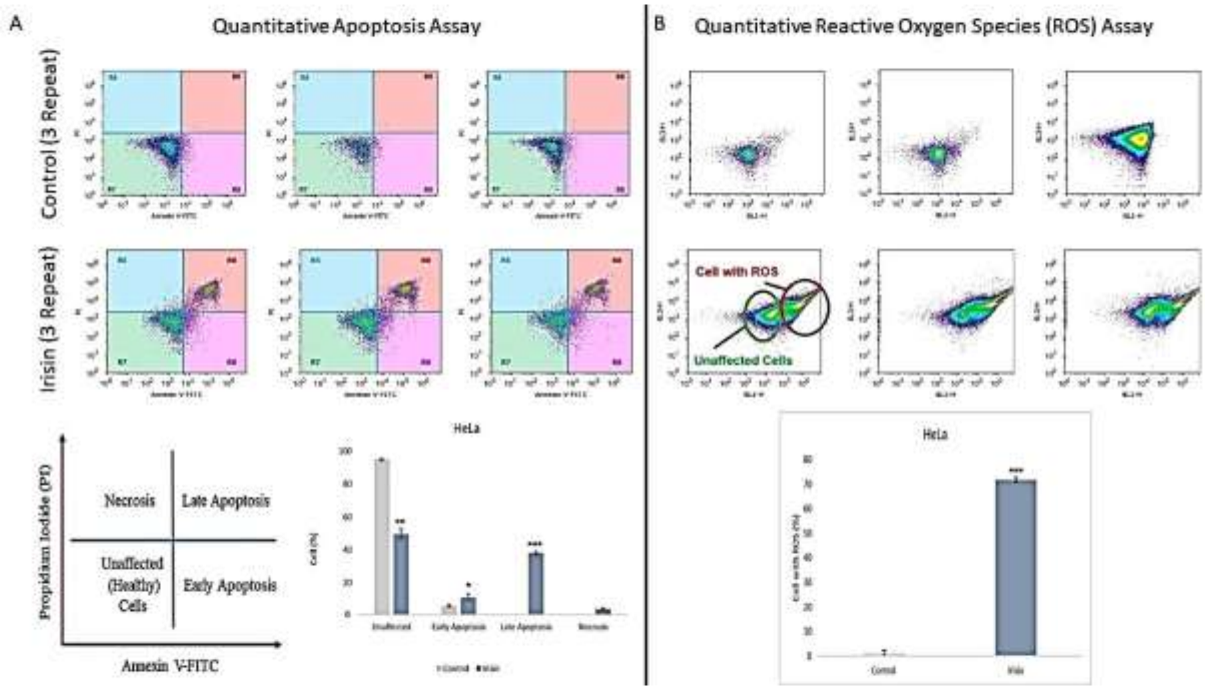


Figure 3. Flow cytometry analyses showing a) the amount of apoptotic cells and b) the amount of ROS-affected cells in the HeLa cell line following the application of 50 nM irisin for 48-h in triplicate (Mean = ±SD, *p<0.05, **p<0.01, ***p<0.005).

Similar to the gene expression analyses, this indicates that irisin suppresses glucose metabolism, leading HeLa cells to undergo apoptosis as a result of ROS impact under hypoxic conditions.

Finally, the changes in the nuclear structures of cells due to the application of 50 nM irisin to the HeLa cell line were compared with the control group and shown under a fluorescence microscope using the Hoechst 34580 staining method (Figure 4).

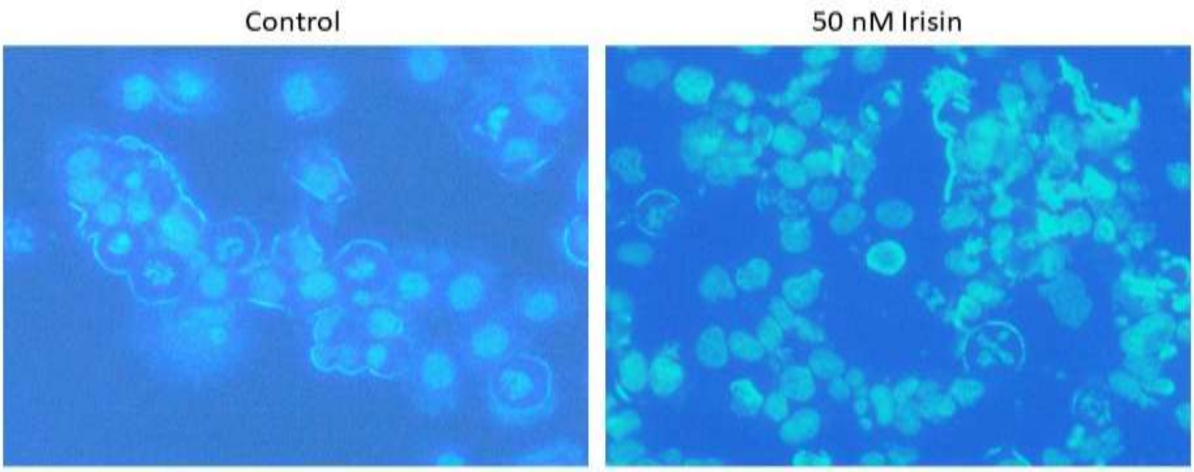


Figure 4. Comparison of the changes in the nuclear structures of cells due to the application of 50 nM irisin to the HeLa cell line with the control group cell line (20× objective).

The dye Hoechst 34580 has a remarkably strong affinity for nucleolar DNA. Since the nuclei in resting cells normally do not condense, Hoechst 34580 binds to DNA poorly and does not cause an excessive brightening. Conversely, in cells that are dividing or going through apoptosis because of DNA damage, the condensation of the nuclear structures diminishes, which makes it possible for Hoechst34580 to bind tightly to the DNA and pass through the weakened links, producing bright fluorescence (Dasari et al., 2013). As seen in Figure 4, the application of 50 nM irisin resulted in the occurrence of bright spots in the nuclear structures of HeLa cells, indicating DNA damage and apoptosis.

CONCLUSIONS

In this study, it was shown that oxidative stress resulting from the suppression of glucose metabolism by irisin leads HeLa cells to undergo apoptosis, while no cytotoxic effects were observed in healthy AML12 cell lines within the 1.56 nM-200 nM dose range, and it even contributed to cell proliferation. Therefore, it is thought that externally administered irisin could have positive effects in combating cervical cancer, particularly HeLa cells. Additionally, since irisin produced by the human body shows complete homology with irisin produced by mice, it is highly likely that this hormone will shed light on successful and rapid results in phase II studies of cervical cancer and other cancers without the need for any post-modification.

REFERENCES

Bostrom, P., J. Wu, M.P. Jedrychowski, A. Korde, L. Ye, J.C. Lo., et al.. 2012. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature, 481: 463-468.

Burd E.M. 2003. Human papillomavirus and cervical cancer. Clinical Microbiology Reviews, 16 (1): 1-17.

Dasari, M., A.P. Acharya, D. Kim, S. Lee, J. Rhea, R. Molinaro, N. Murthy. 2013. H-gemcitabine: A new gemcitabine prodrug for treating cancer. Bioconjugate Chemistry, 24: 4-8.

Gannon, N.P., R.A. Vaughan, R. Garcia-Smith, M. Bisoffi, K.A. Trujillo. 2015. Effects of the exercise-inducible myokine irisin on malignant and non-malignant breast epithelial cell behavior in vitro. International Journal of Cancer, 136: E197-E202.

Gasinska, A., A. Janecka, A. Adamczyk, D. Słonina. 2013. How tumour cells respirate? Nowotwory Journal of Oncology, 63: 124-131.

Hofmann, T., U. Elbelt, A. Stengel. 2014. Irisin as a muscle-derived hormone stimulating thermogenesis–a critical update. Peptides, 54: 89-100.

Huh, J.Y., G. Panagiotou, V. Mougios, M. Brinkoetter, M.T. Vamvini, B.E. Schneider. 2012. FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise. Metabolism, 6: 1725-1738.

Józwiak, P., A. Lipinska. 2012. The role of glucose transporter 1 (GLUT1) in the diagnosis and therapy of tumors. Postepy Higieny i Medycyny Doswiadczalnej, 66: 165-174.

Jones, H.W. 1997. Record of the first physician to see Henrietta Lacks at the Johns Hopkins Hospital: History of the beginning of the HeLa cell line. American Journal of Obstetrics and Gynecology, 176 (6): s227-s228.

King, D., D. Yeomanson, H.E. Bryant. 2015. PI3King the lock: Targeting the PI3K/Akt/mTOR pathway as a novel therapeutic strategy in neuroblastoma. Journal of Pediatric Hematology and Oncology, 37 (4): 245-51.

Kong, G., Y. Jiang, X. Sun, Z. Cao, G. Zhang, Z. Zhao, Y. Zhao, Q. Yu, G. Cheng. 2017. Irisin reverses the IL-6 induced epithelialmesenchymal transition in osteosarcoma cell migration and invasion through the STAT3/Snail signaling pathway. Oncology Reports, 38: 2647-2656.

Köhl, R., J. Zhou, B. Brüne. 2006. Reactive oxygen species attenuate nitric-oxide-mediated hypoxia-inducible factor-1alpha stabilization. Free Radical Biology and Medicine, 40 (8):1430-42.

Liberti, M.V., J.W. Locasale. 2016. The warburg effect: How does it benefit cancer cells? Trends in Biochemical Sciences, 41: 211-218.

Lin, Y., K. Xue, Q. Li, Z. Liu, Z. Zhu, J. Chen, et al.. 2021. Cyclin-dependent kinase 7 promotes Th17/Th1 cell differentiation in psoriasis by modulating glycolytic metabolism. Journal of Investigative Dermatology, 141 (11): 2656-2667.e11.

Masson, N., R.S. Singleton, R. Sekirnik, D.C. Trudgian, L.J. Ambrose, M.X. Miranda, et al. 2012. The FIH hydroxylase is a cellular peroxide sensor that modulates HIF transcriptional activity. EMBO Reports, 13 (3): 251-7.

Moon, H.S., C.S. Mantzoros. 2014. Regulation of cell proliferation and malignant potential by irisin in endometrial, colon, thyroid and esophageal cancer cell lines. Metabolism, 63: 188-193.

Park, S.T., B.R. Kim, S.H. Park, J.H. Lee, E.J. Lee, S.H. Lee, et al.. 2014. Suppression of VEGF expression through interruption of the HIF−1α and akt signaling cascade modulates the anti−angiogenic activity of DAPK in ovarian carcinoma cells. Oncology Reports, 31 (2): 1021-9.

Roberts, M.D., D.S. Bayless, J.M. Company, N.T. Jenkins, J. Padilla, T.E. Childs. 2013. Elevated skeletal muscle irisin precursor FNDC5 mRNA in obese OLETF rats. Metabolism, 62: 1052-1056.

Shao, L., H. Li, J. Chen, H. Song, Y. Zhang, F. Wu, W. Wang, W. Zhang, F. Wang, H. Li, et al.. 2017. Irisin suppresses the migration, proliferation, and invasion of lung cancer cells via inhibition of epithelial-to-mesenchymal transition. Biochemical and Biophysical Research Communications, 485: 598-605.

Shi, G., N. Tang, J. Qiu, D. Zhang, F. Huang, Y. Cheng, K. Ding, W. Li, P. Zhang, X. Tan. 2017. Irisin stimulates cell proliferation and invasion by targeting the PI3K/AKT pathway in human hepatocellular carcinoma. Biochemical and Biophysical Research Communications, 493: 585-591.

Tekin, S., Y. Erden, S. Sanda, B. Yilmaz. 2015. Is irisin an anticarcinogenic peptide? Medical Sciences, 4: 2172-2180.

Timmons, J.A., K. Baar, P.K. Davidsen, P.J. Atherton. 2012. Is irisin a human exercise gene? Nature, 488: E9-10.

Wang, S., J. Pan. 2016. Irisin ameliorates depressive-like behaviors in rats by regulating energy metabolism. Biochemical and Biophysical Research Communications, 474: 22-28.

Zhang, T., S. Guo, X. Zhu, J. Qiu, G. Deng, C. Qiu. 2020. Alpinetin inhibits breast cancer growth by ROS/NF-κB/HIF-1α axis. Journal of Cellular and Molecular Medicine, 24 (15): 8430-40.

Zhang, Y., Q. Mu, Z. Zhou, H. Song, Y. Zhang, F. Wu, M. Jiang, et al. 2016. Protective effect of irisin on atherosclerosis via suppressing oxidized low density lipoprotein induced vascular inflammation and endothelial dysfunction. PLoS One, 11: e0158038.

MULTIDISCIPLINARY ANALYSES OF FRACTURED AQUIFERS IN A PRECAMBRIAN BASEMENT OF AMZIGGAR, AGADEZ REGION (NORTH NIGER)

Illias Alhassane[1], Abdou Babaye Maman Sani[2], Issa Malan S.Souleymane[3]*

[1]Department of Geology, Faculty of Science and Techniques, Agadez University, Agadez, Niger.
[2]Department of Geology, UMR SERMUG, Faculty of Science and Techniques, Dan Dicko Dankoulodo University, Maradi, Niger.
[3]Department of Geology and Environment, Faculty of Sciences and Techniques, Zinder, University, Zinder, Niger.
Corresponding author e-mail: illiasalhassane@gmail.com

ABSTRACT

Amziggar is located in Tabelot municipality west edge of Aïr massif, arid region. The geological formations are constitued by the basement. The water resources is represented by the alluviums aquifers which often dry up before the return of rainy season, with the development of gold-mining activities and its concomitant attraction of local population see national and international have highly exert pressure on these weak groundwater resources. The objective of this study is to determine the fractured and/ or altered areas favorable for drilling hydraulic borholes. The methodological approach, which based on the combined methods of Remote sensing and GIS and field work are allowed to cartograhy and mapping the fractures network. The fracture map produced shows that the main fractures are oriented N0°-N10°, N90°-N100°, N130°-N150°, and N40°-N50° directions.This map will be a precious tool in future groundwater research for Tabelot municipality.
Keywords: DEM, GIS, fractured aquifers, Remote sensing, Arid region, Agadez

INTRODUCTION

In fractured zones, fracking and weathering are the two main conditions of aquifer existence (Engalenc, 1978). In West Africa, several studies have shown great interest in the study of fracking in the identification of discontinuous aquifers including in Ivory Coast (Sokeng et *al*., 2014; Koudou et *al*., 2010; Assoma et *al*., 2013; Kouakou et *al*., 2014), in Burkina Faso (Nakolendousse, 1991; Savadogo, 1984; Faye et *al*., 2023) and in Niger (Corgne et *al*., 2010; Alhassane et *al*., 2018; Babaye et *al*., 2021; Alhassane et *al*., 2023) . These studies have provided very satisfactory results in the determination of discontinue aquifers of basement, mainly the areas where drilling is being carried out.
The area of Amziggar, in the massif of Aïr, is an arid area located in the basement area by excellence and has great difficulty in supplying drinking water. Water resources are represented by alluvial aquifers that dry up before the return of rains(Alhassane et *al*., 2018;

Alhassane et *al*., 2019). With the development of semi-mechanized gold mining activities, this water resources available are heavily stressed for water supply to the populations and for gold mining activities. Moreover, this is added an increase in the population due to the activities of gold comes from various horizons of the country or other nationalities. The area of Amzzigar has become a hub in the economy of Agadez region and for the municipality of Tabelot in particular.

Several tantative of implantation of waters supply was realized by the privates and the the holders of licences of gold mining in the area. However, a significant failure rate is reported from several drilled boreholes. Also, other positive boreholes dry in less than one year of activities. Thus, it is necessary to carry out a study in order to determine areas favorable for drilling borehols. This is achieved by the determination of fractured and/or altered areas. It is in this context that the present study is part of which aims to determine the fractured zones and the favorable areas for wells hydraulic works in Amziggar area. It will be used the methods of sapatial analyses including the Geographic Information Systems (GIS) and Remote sensing.

The Digital Elevation Model (DEM) offers various advantages in the mapping of fractured areas as well as the characterization of the fractured and altered reservoirs of the basement (Cardona et *al*., 2004; Durand, 2005; Ta et *al*., 2008; Alhassane et *al*., 2018).

MATERIAL AND METHODS

Location of study area

The area of Amziggar is the western part of the rural municipality of Tabelot, located at 200 km northwest of the head town of the Department of Tchirozerine and 160 km north at the chief town of Agadez Region. This area belongs to the western flank of Bagzam Mount and its space is dominated by small mountain massifs more or less raked off the mount of "Amziggar" from which the locality gets its name. This municipality is located between 8°51’40’E and 8°58’10’E of east longitude and 17°32’20’’ N and 17°36’40’' N of north latitude (Fig.1).

The climate is semi-arid, of Sahelo-Saharan type with high thermal amplitudes. Four seasons are distinguished in the year: (i) The cold season that goes from november to march. This is the season of harmattan, a dry wind that blows continuously bringing dry mists (Morel, 1983) (Morel and Giazzi, 2001). It is characterized by a drop in temperatures. (ii) The warm season that extends from april to june. This is the season when the intertropical front (ITF) gradually rises northward and the monsoon (humid air) slips under the harmattan. (iii) The rainy season lasts from july to september; and the intermediate season is rather short but difficult for farmers to overcome. Annual averages of low rainfall are always below 200 mm (Morel, 1983). The rains are irregular and poorly distributed in time and space.

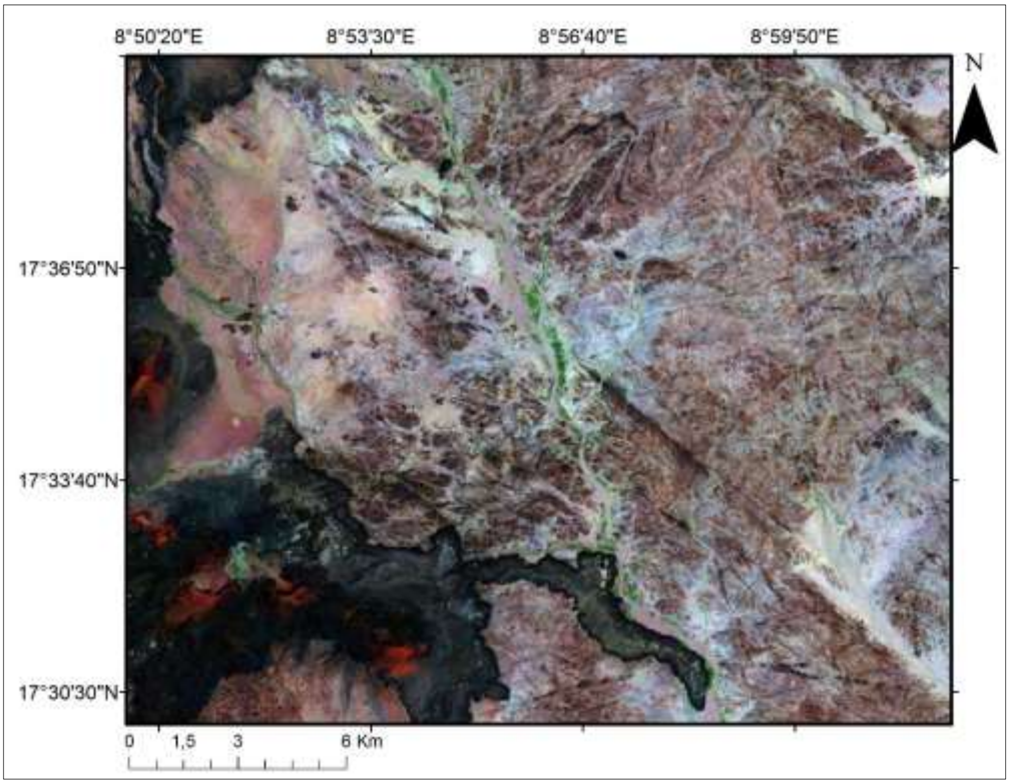


Figure 1. Location map of study area

Geological and hyrogeological context

The Aïr massif is a south-eastern appendix of the Tuareg shield (fig.2). It consists of three main domains that are from West to East: (i) the Assodé domain, formed by two N-S accidents: the Arlit fault in the West and the "Raghane" accident in the East; (ii) the Barghot domain; and (iii) the Aouzegueur domain (Liégeois et *al*., 1994; Liégeois et *al*., 2005; Liégeois et *al*., 2013). At the end of the phase of convergence between the West African craton and the Saharan meta-craton, around 670 Ma, the domains of Aouzegueur and Barghot are charred and attached to the East-Saharan craton (Black et *al*., 1985; Black et *al*., 1994) while the Assodé estate moved by dextrous sliding along the "Raghane" accident, northward, to the upper Pan-African.

The geological formations of Aïr Massif include (Black et *al*., 1979; Black et *al*., 1985) of eburnian gneiss containing amphibolites, quartzites, diopside marbles, granitoid of Precambrian anatexia related to pan-African tectonics and sub-volcanic and hyperalkaline volcanic complexes ("younger granites") (Moreau et *al*., 1994; Pouclet et *al*., 1994; Pouclet and Durand, 1985; Pouclet et *al*., 2006) with annular structures intersecting the whole, and which were set up in the Ordovician and Silurian (Black et *al*., 1985) (Pouclet et *al*., 1994) (. According to Joseph, (1991) four accident families affects the massif according to the directions NW-SE, N80°, N45° and N120°-N150°.

The aquifers of the alterites are located in the altered zones of the basement, they are mainly captured by cemented wells with large diameter and their average depth is about 22 m. The waters are of an average salinity, for most of the works. Alluvial aquifers are located on the edges, and at the bottom of the koris which generally cut the young massifs to the foothills from which the springs and gueltas are found (Joseph et *al*., 1990; Joseph, 1991; Gallaire, 1995). These aquifers are captured by shallow wells (6 to 18 m). The waters of these aquifers have low salinity.

The site is located in the massif of Aïr, it is mainly based on the crystalline basement, where the geological framework is represented by magamatic and crystallophyllic formations (Fig.2). Thus, the resources of water in the sub-region are confined to alluvial streams (valleys) representing shallow alluvial aquifers captured by cemented wells. The second reservoir is formed by the fractured, cracked and or altered formations of the basement.

As for the catchment works, they are constituted mainly by cemented wells of shallow depth (22 m) capturing the alluvial layer dependent on rainfall. The water in these aquifers is poorly mineralized. They are used for the supply of drinking water to populations, abreveument of the livestock and in market activities. They are also represented in recent years by drilling to capture the discontinuous aquifers of the base with more or less important flows around 6 m^3/h. In some places, the water of the aquifers of the base is highly mineralized and unsuitable for any activity.

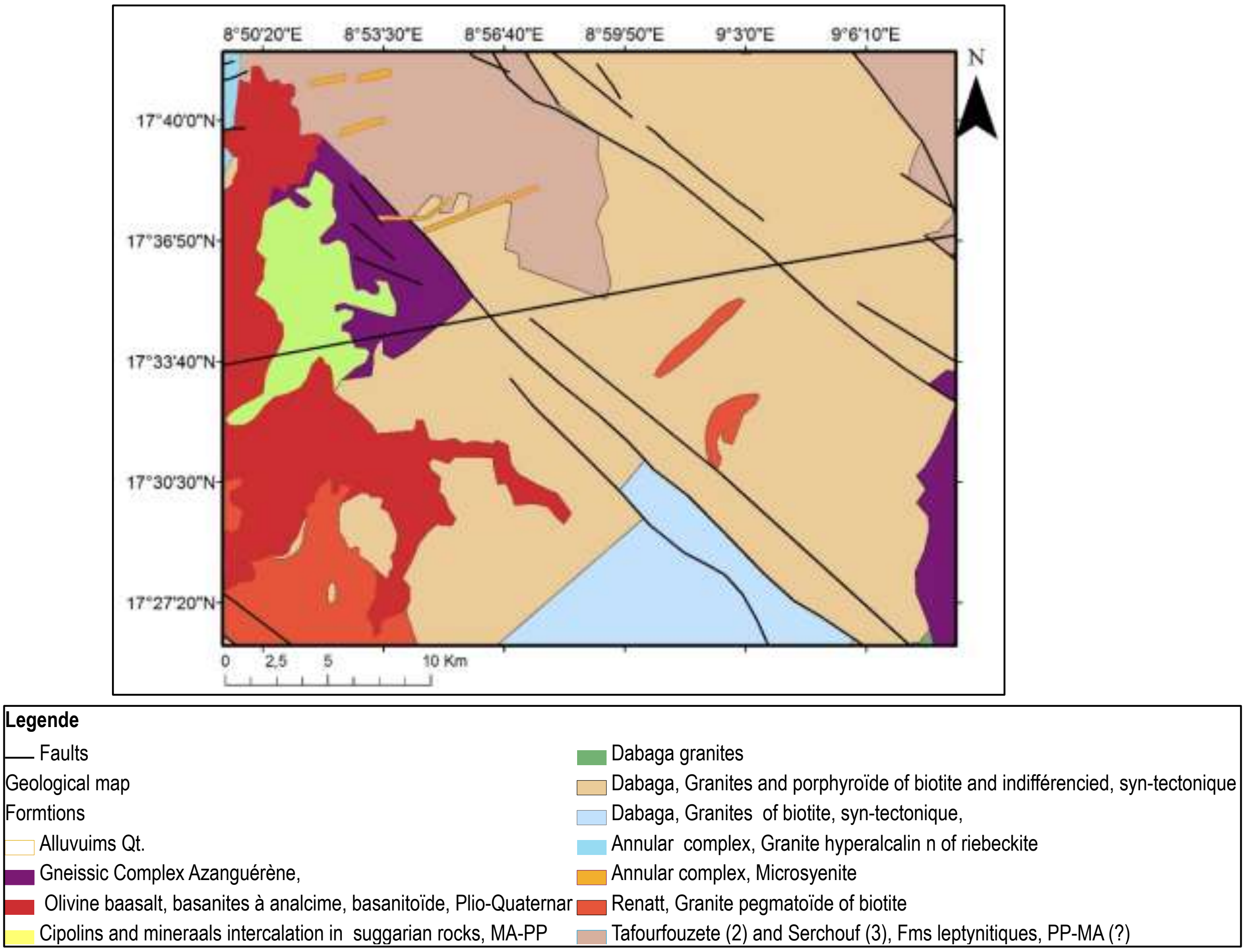


Figure 2. Geological map of the study area

METHODOLOGY

The cartographic database used for this study mainly includes a Digital Elevation ModelModel (DEM, resolution 30 m, scenes: 17-008 and 17-009, SRTM 30 M, NASA, USGS/2011). A topographic map of the Elmeki sector (Marchaud et *al*., 1961) (sheet Elmeki NE-32-IV) at 1/200 000e and a geological map of the Air (Black et *al*., 1979) were also used at 1/500 000e .

The DEM, which was image-to-image corrected, was performed from four calibration points from the area corrected ETM^+ bands. The degree 1 polynomial transformation and a re-sampling by the cubic convolution method were used for these four calibration points. The average RMS (Error Refractive Mean) for the DEM image is 0.218 m and compared to half pixel (15m), these errors are smaller than this one, so correction is acceptable (Corgne et *al.*, 2010; Koita et *al*., 2010).

After the pretreatment phase, the Digital Elevation Model (DEM) was subjected to various treatments including the technique of the stompage. This classic mapping technique, is to represent the effect of the relief by illuminating the DEM in a defined direction and thus creating shadows forming a monochrome image (Donnay, 2000). In this study, the DEM was estomping in four directions (N045°, N090°, N135° and N180°) to accentuate the structural directions perpendicular to them (NW-SE, N-S, NE-SW and E-W). This will allow the lineaments to be identified and plotted manually (Babaye, 2012; Sokeng et *al*., 2014; Alhassane et *al*., 2018; Babaye, et *al*., 2021) (Fig.3).

The lineaments highlighted from the DEM estompage were confronted and superimposed on the accidents represented on the geological map (Black et *al*., 1979) to gave a structural significant of lineaments for the validation data. However, on the geological map the density of faults is very low and it has been supplemented by field work. Subsequently, a field survey with outcrops and hydrogeological observations was carried out: (i) Type of aquifer and depth of the captured water table, (ii) identification of flood and erosive zones; (iii) location of possible pollution hotspots (cemeteries-livestock run and pass area); and (iv) information on the hydrographic network.

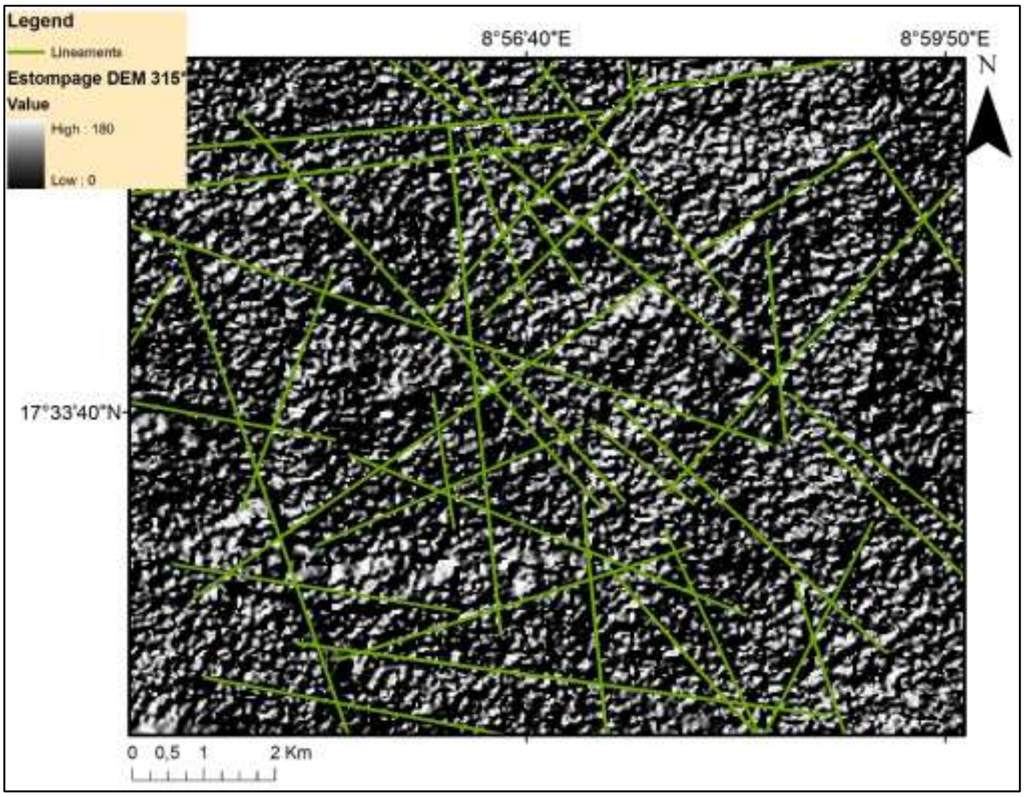


Figure 3. Estompage of DEM in 315 °

RESULTS AND DISCUSSION

Directional distribution of fractures

The four main fracture directions in number with the highest percentages, (Table 1), are N0°-N10°, N90°-N100°, and N160°-N180°, with respective percentages: 16%, 11%, and 9%. Then comes the directions N140°-N150°, N100°-N110°, and N130°-N150°, N150°-N160°, N120-N130°which have only percentages of 7% to 5%.

With regard to the cumulative lengths of fractures (Table 1), four main directions are distinguished: N130°-N140°, N40°-N50°, N140°-N150°, N160°-N170°, with the respective percentages being: 13%, 10%, 10% and 8%. Finally, the lowest percentages are found in N100°-N110°, N170°-N180° and N130°-N140°, with 7% and 6%, respectively.

Table 1: Percentages of fracture directions, in number and cumulative lengths, from the DEM estompage.

Supports	Directions	% number of fractures	Directions	% cumulatives lengt of fractures
MNT (Estompage)	N0°-N10°	16%	N130°-N140°	13%
	N90°-N100°	11%	N40°-N50°	10%
	N160°-N170°	9%	N140°-N150°	10%
	N170°-N180°	9%	N160°-N170°	8%
	N140°-N150°	7%	N100°-N110°	7%
	N100°-N110°	7%	N170°-N180°	6%
	N130°-N150°	5%	N130°-N140°	6%
	N150°-N160°	5%	Others	< 5%
	N120°-N130°	5%		
	Others	< 5%		

Synthesis of fractured mapping

The summary of the results of the statistical processing of fracking data (fig. 4) shows that the following directions: N0°-N10°, N90°-N100° and N160°-N180° are dominant followed by those that are secondary, oriented: N140°-N150°and N100°-N110°.

The cumulative fracture lengths (Fig.5b) are more important in the directions N130°-N140°, N140°-N150°, and N40°-N50°, corresponding to those of the mega fractures. Next are directions N160°-170° and N100°-N110°, with little significant cumulative length for secondary fractures.

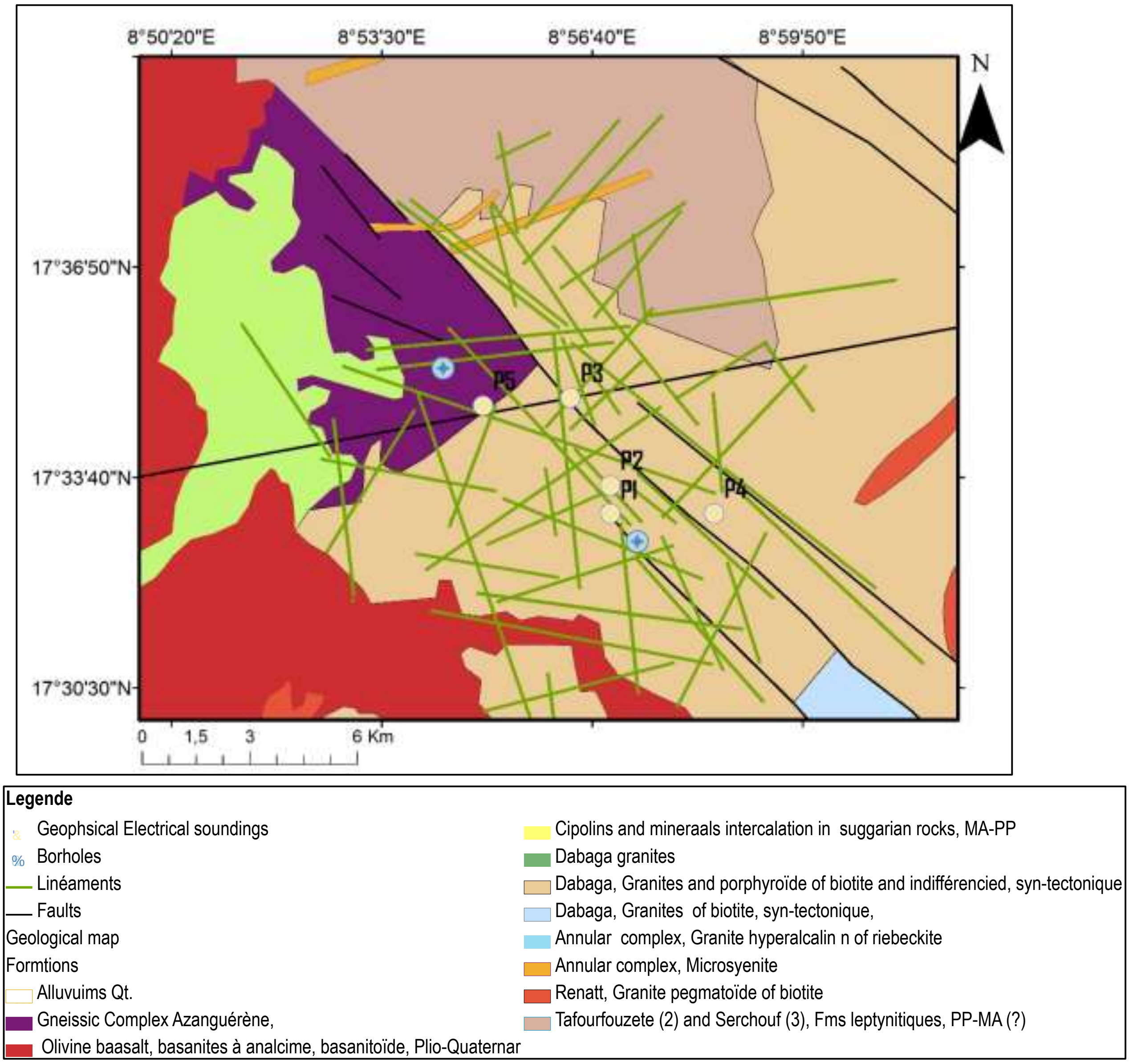


Figure 4. Fracturation Map of study area

DISCUSSION

In the fracking network mapping, the DEM blurring has made it possible to highlight the usefulness of this tool in the characterization of fracturation in the basement zones. Various work has used the DEM in identifying discontinuous aquifers of the basement (Durand, 2005; Corgne et *al.*, 2010; Otchoumou et *al.*, 2012; Sokeng et *al.*, 2014; Alhassane et *al.*, 2018; Babaye et *al.*, 2021).

The direction N0°-N10° is the most abundant and represents the shear of "Raghane" crossing the Aïr on the north-south direction wide from 5 to 10 km over more than 400 km (Liégeois et *al.*, 1994). In the area of Timia, central Aïr, this direction is also well represented and it is the most important (Alhassane et *al.*, 2018) .

Accidents N90°-N100° and N160°-N180° are the most numerous after class N0°-N10° in the Amziggar area. The former are observed throughout the study area and control the direction of most watercourses in the orientation zone (N70°-N80°, N90°-N100°). In Timia area, most courses are in this direction (Alhassane et *al*., 2018). According to (Joseph, 1991), the accidents N70°-N80° affecting not only the base but also the sedimentary formations of the western edge of the massif of the Aïr, can provide a possible hydraulic transfer.
Accidents N160°-N180° are the majority of directions for quartz and quartzite veins in the Central Aïr (Alhassane et *al*., 2018). the presence of N160°-N170° accidents is evidenced in the Téfidet trough (Ahmed et *al*., 2017).
The N130°-N140° directional fractures represent the largest class in length accumulated in the Amziggar sector, constituting mega-lineaments observable over great distances (Black et *al*., 1979). Also, they were observed mainly on the relics of the Pan-African base flush on the eastern, western and central edges of the Timia area (Alhassane et *al*., 2018). These fracture bundles played a significant role in the structuring of the Tefidet trough (Black et *al*., 1979; Ahmed et *al*., 2017). The N140°-N150° direction fractures give the same percentage as the N130°-N140°(10%) in length cumulative. Thus, they are of the same family of accidents. In the case of N40-N50 accidents, it is carried by numerous rhyolitic and gabbro dykes (Black et *al*., 1979). Finally, accidents N160°-N170°, this family is observed in the Téfidet trough (Ahmed et *al*., 2017).
Various works carried out in the West African base based on satellite images analysis, in Niger (Alhassane et *al*., 2018; Babaye et *al*., 2021), in Côte d'Ivoire (Lasm, 2000; Kouassi et *al*., 2012; Kouamé et *al*., 2014; Koudou et *al*., 2014) and in Burkina Faso (Nakolendousse; 1991; Koussoubé, 2003; Faye et al., 2023) have improved knowledge of the fractured aquifers of the base.

CONCLUSIONS

The analysis and exploitation of the Digital Model elevation (DEM) has made possible to identify and map the fracture network in the Amziggar sector. They are mainly oriented towards N0°-N10°, N90°-N100° and N160°-N180° in number of fractures and N130°-N140°, N140°-N150°and N40°-N50° in length cumulated. These fractures are not enough to constitute an aquifer. However, these structures in conjunction with other additional hydrogeological information may help to identify areas favourable for the establishment of drilling in the Amziggar area. Geophysical studies will confirm these structures in evidence as well as their water saturation.

REFERENCES

Abdou Babaye, M.S. 2012. Assesment of groundwater resources in the Dargol basin (Liptako-Niger). PhD thesis, Liège, University de, 244p.

YAcouba A, Moussa K. and M Harouna. 2016. Tectono-magmatic reactivation of Téfidet Cretaceous trough during Cenozoic (Aïr, Niger) Bull. Soc. géol. France, 2016, t. 187, no 2, pp. 73-83

Alhassane, I., Sani, A.B.M., Souleymane, I.M.S. and Wagani, I. 2023. Application of Spatial Techniques for the Identification of Discontinuous Aquifers of the Basement in Semi-Arid Environment: A Case of Bagzan Mount Plateau, Aïr Massif (North, Niger). Journal of Water Resource and Protection, 15, 581-596.

https://doi.org/10.4236/jwarp.2023.1511032

Alhassane, I., Sani, A.B.M. and Souleymane, I.M.S. 2023. Contribution of Geophysical Prospecting (1D) to Characterize a Precambrian Basement Aquifers Agadez Region (North, Niger). International Journal of Geosciences , 14, 1177-1191. https://doi.org/10.4236/ijg.2023.1411060

Alhassane, I., Maman Sani, A.B., Issoufou, S., Bachir, S.M., Boureima, O. 2018. Contribution of ETM+ images and the Digital Elevation Model (DEM) to the fracturation mapping In mountainous region: Timia sector (Aïr Massif , North of Niger). European Scientific Journal March 2018 edition Vol.14, No.9 ISSN: 1857 – 7881 (Print) e-ISSN 1857- 7431.

Alhassane, I., Sani, A.B.M., Issoufou, S., Souleymane, I.M.S. 2019. Hydrogeochemical characterization of Timia Basin aquifers (Aïr Massif, North, Niger). European Scientific Journal October 2019 edition Vol.15, No.30 ISSN: 1857-7881 (Print) e-ISSN 1857-7431.

Assoma, V.T., Kouame, F.K., Saley, B.M., De Dreuzy, J.-R. 2013. Using Remote Sensing Data and a Fluid Flow Model for Groundwater Exploration in Fractured Rock of Koue Watershed (Western Côte d'Ivoire). Int. j. eng. sci 2, 454–462.

Babaye, M.S.A., Hantchi, K.D., Wagani, I., Ousmane, B.I., Sandao, I. 2021. Multi-Criteria Analysis Using Remote Sensing and GIS: Insight into Groundwater Potential of the Fissured Aquifers in the Liptako Socle (Southwestern Niger). Journal of Water Resource and Protection 13, 881–899.

Black, R., Ba, H., Ball, E., Bertrand, J.M., Boullier, A.M., Caby, R., Davison, I., Fabre, J., Leblanc, M., Wright, L.I. 1979. Outline of the Pan-African geology of adrar des Iforas (Republic of Mali). Geologische Rundschau 68, 543–564.

Black, R., Lameyre, J., Bonin, B. 1985. The structural setting of alkaline complexes. Journal of African Earth Sciences (1983) 3, 5–16.

Black, R., Latouche, L., Liégeois, J.-P., Caby, R., Bertrand, J.-M. 1994. Pan-African displaced terranes in the Tuareg shield (central Sahara). Geology 22, 641–644.

Babaye M.S., Illias A. Issa M.S., Boukari O.I., and M. Konaté. 2021. Synergistic approach between spatial technique and field data for the identification of fractured basement aquifers in semi-arid environment (Liptako, South-west Niger). Bulletin de l'Institut Scientifique, Rabat, Section Earth Sciences, 2021, n° 43, 53–68.

Cardona, A., Rivera, J.J.C., Alvarez, R.H., Castro, E.G. 2004. Salinization in coastal aquifers of arid zones: An example from Santo Domingo, Baja California Sur, Mexico. Environmental Geology 45. https://doi.org/10.1007/s00254-003-0874-2

Corgne, S., Magagi, R., Yergeau, M., Sylla, D. 2010. An integrated approach to hydro-geological lineament mapping of a semi-arid region of West Africa using Radarsat-1 and GIS. Remote Sensing of Environment 114, 1863–1875.

Durand, V. 2005. Multidisciplinary research to characterize two fractured aquifers: the mineral waters of Plancoët in a metamorphic context, and of Quezac in a carbonated environment. Pierre and Marie Curie-Paris VI University. 255P.

Donnay J. P. 2000. Bulletin of the Geographical Society of Liège, 38, 2000/1, pp 107-119.
Engalenc M. 1978. Method for the study and research of groundwater in crystalline rocks in West Africa. Inter-African Committee on Water Studies, (CIEH), 318p.

Faye Moussa Diagne, Biaou Angelbert Chabi, Doulkom Palingba Aimé Marie, Koita Mahamadou, and Yacouba Hamma. (2023). Contribution of Remote Sensing and Geophysical Prospecting (1D) to the Knowledge of Groundwater Resources Burkina Faso." American Journal of Water Resources, vol. 11, no. 2 (2023): 49-64. doi: 10.12691/ajwr-11-2-2.

Joseph, A. 1991. The Aïr, «water tower» of the desert strip of the Tenerés (Niger). Journal of Alpine Geography 79, 71–86.

Joseph, A., Aranyossy, J.F., Kanta, I. 1990. Recharges and palerecharges of discontinuous aquifers of Aîr basement (Niger). Geodinamica Acta 4, 185–197.

Koita, M., Jourde, H., Ruelland, D., Koffi, K., Pistre, S., Savane, I. 2010. Mapping of regional accidents and identification of their role in the subsurface hydrodynamics in the basement areas. Case of the region of Dimbokro-Bongouanou (Côte d'Ivoire). Hydrological sciences journal 55, 805–820.

Kouakou, K.E.G., Lasm, T., Sombo, B.C., Ta, M.Y., Baka, D., Kouadio, K.E. 2014. Contribution of geophysics in structural study and in identification of fissured aquifers in the department of Dabakala (North Central of Côte d'Ivoire). International Journal of Innovation and Applied Studies 8, 1752.

Kouamé, A.N., Amani, M.K., Gnaboa, R., Traoré, K.S., Houenou, P.V. 2014. Analysis of hydrological phenomena in an urbanized watershed: the case of the city of Yamoussoukro (central of Côte d'Ivoire). LARHYSS Journal P-ISSN 1112-3680/E-ISSN 2521-9782.

Kouassi, A.M., Ahoussi, K.E., Yao, K.A., Ourega, W., Yao, K.S.B., Biemi, J. 2012. Productivity analysis of the cracked aquifers in the N'zi-Comoé region (Central-Est of Côte d'Ivoire). LARHYSS Journal P-ISSN 1112-3680/E-ISSN 2521-9782.

Koudou, A., Assoma, T.V., Adiaffi, B., Ta, M.Y., Kouame, K.F., Lasm, T. 2014. Statistical and geostatistical analyses of fracking extracted from the ASAR ENVISAT imagery of the South-East of Côte d'Ivoire. LARHYSS Journal P-ISSN 1112-3680/E-ISSN 2521-9782.

Koudou, A., Kouamé, F.K., Ta, M.Y., Saley, M.B., Jourda, J.P., Biemi, J. 2010. Contribution of ETM+ data from Landsat, multi-criteria analysis and a GIS to the identification of aquifer potential areas in the base zone of the N'Zi watershed (Côte d'Ivoire). Photo Interpretation European Journal of Applied Remote Sensing 46, 98.

Koussoubé, B. 2003. The different signatures of the crystalline basement fractures in the Sahel-Sudan zone of Burkina Faso (Bidi watershed, Yatenga province). Remote sensing 3, 419–427.

Gallaire, R., 1995. Hydrology in high-altitude sub-desert: the case of Aïr (Niger). Paris 11.

Lasm, T. 2000. Hydrogeology of the fractured basins: statistical and geostatistical analyses of fracking and hydraulic properties; application to the mountain region of Côte d'Ivoire (domaine archéen). Thesis Poitiers University.

Liégeois, J., Benhallou, A., Azzouni-Sekkal, A., Yahiaoui, R., Bonin, B. 2005. The Hoggar swell and volcanism: reactivation of the Precambrian Tuareg shield during Alpine convergence and West African Cenozoic volcanism. Special papers-Geological Society of America 388, 379.

Liégeois, J.-P., Abdelsalam, M.G., Ennih, N., Ouabadi, A. 2013. Metacraton: nature, genesis and behavior. Gondwana Research 23, 220–237.

Liégeois, J.-P., Black, R., Navez, J., Latouche, L. 1994. Early and late Pan-African orogenies in the Aïr assembly of terranes (Tuareg shield, Niger). Precambrian research 67, 59–88.

Moreau, C., Demaiffe, D., Bellion, Y., Boullier, A.-M., 1994. A tectonic model for the location of Palaeozoic ring complexes in Aïr (Niger, West Africa). Tectonophysics 234, 129–146.

Morel, A. 1983. Erosion and sbdimentation in the Air Massif (southern Sahara): palaeoclimatic interpretation of the middle terrasse.

Morel, A., Giazzi, F. 2001. Is there a mountain specific of the massif of Aïr, south of the Sahara? Tropical spaces 16, 263–270.

Nakolendousse, S. 1991. Method of assessing the productivity of aquifer sites in Burkina Faso: geology, geophysics, remote sensing. Grenoble 1.

Otchoumou, K.F.E., Bachir, S.M., Etienne, A.G., Issiaka, S. 2012. Contribution of remote sensing and GIS in the identification of groundwater resources in the region of Daoukro (Centre-Est of Côte d'Ivoire). International Journal of Innovation and Applied Studies 1, 35-53.

Pouclet, A., Ahmed, Y., Baubron, J.-C., Bellon, H., Morel, A. 1994. Age and establishment of the Cenozoic volcanism in the graben of the Téfidet (rift system of eastern Niger). Comptes Rendus de l'Académie des Sciences (France), Série II 318, 683–690.

Pouclet, A., Doumbia, S., Vidal, M. 2006. Geodynamic setting of the Birimian volcanism in central Ivory Coast (western Africa) and its place in the Palaeoproterozoic evolution of the Man Shield. Bulletin de la Société Géologique de France 177, 105–121.

Pouclet, A., Durand, A. 1994. Cenozoic brittle structures from volcanic and neolecionic phenomena in the northwest of Lake Chad (Niger oriental).

Pouclet, A., Lee, J.-S., Vidal, P., Cousens, B., Bellon, H. 1994. Cretaceous to Cenozoic volcanism in South Korea and in the Sea of Japan: magmatic constraints on the opening of the back-arc basin. Geological Society, London, Special Publications 81, 169–191.

Savadogo, N. 1984. Geology and hydrogeology of the crystalline base of Upper Volta: Regional study of the Sissili watershed. University of Science and Medicine.

Sokeng, V.-C.J., Kouamé, K.F., TA, M.Y., Saley, M.B., Kouame, K. 2014. Automatic extraction of the lineaments on satellite images by neural networks: contribution to the structural mapping of the precambrian base of the Bondoukou region (north-east of Côte d'Ivoire). Revue Scientifique Internationale de Géomatique 1.

Sorokoby, V.M., Saley, M.B., Kouamé, F.K., M'Moi, E., Djagoua, V., Bernier, M., Kouadio, A., Biémi, J. 2010. Use of Landsat ETM+ and SIRS images for the linear and thematic mapping of Soubré-Méagui (South-West Côte d'Ivoire). Revue Teledetection, recherche et application.

Ta, M.Y., Lasm, T., Jourda, J.P., Kouamé, K.F., Razack, M. 2008. Geological accident mapping by Landsat-7 ETM+ satellite imagery and analysis of the fracture networks of the precambrian basement of the Bondoukou region (northeast of Côte d'Ivoire). Télédétection 8, 119-135.

STUDY OF WATER TRANSFER IN SOIL. EFFECTS OF TEXTURE AND SALINITY

Karou Samira[1], Benkhelifa Mohammed[1]

[1]Department of Soil Science and Environment, Higher School of Agronomy, Mostaganem (ESAM), Algeria

Corresponding author e-mail: karousamira2@gmail.com

ABSTRACT

Soil is a key component of the planet and plays a crucial role in achieving the United Nations Sustainable Development Goals and addressing the challenge of land degradation neutrality. Salinity and sodicity are among the most significant challenges faced by irrigated agriculture worldwide Due to the scarcity of fresh water, highly saline groundwater has become the primary source of irrigation. Saline water is used to irrigate cultivated lands in various parts of the world, especially in arid and semi-arid regions that cover 41% of the Earth's surface (UNDP 1997), despite the poor quantity and quality of this resource. Salts are naturally present in soil and groundwater. However, when the levels of soluble salts in the soil or water exceed natural concentrations, it can lead to salinity, posing significant risks to health and reducing plant productivity. Globally, approximately 0.9 × 10^9 hectares are affected by varying levels of salinity and sodicity, and this soil degradation is progressively worsening over time. The widespread presence of saline soils and waters, along with their current and future intensive use in agriculture, necessitates immediate attention and concerted efforts to improve their productivity, which is generally very low under routine management conditions.

To address this issue, gaining a deeper understanding of how salinity and sodicity affect the hydraulic properties of soils, along with their interactions with other soil characteristics, is essential. An energy-based approach to monitoring salinity and sodicity is crucial for comprehending water movement through the soil. This method considers soil texture and the gradients of salinity and sodicity within the soil profile. It serves as a vital tool for understanding how varying levels of salinity and sodicity impact soil hydraulic properties. Consequently, soil hydraulic conductivity is a key factor for the restoration of desert lands and the planning of irrigation projects. This understanding is crucial for assessing soil degradation, restoring affected soils, and effectively planning their conservation and optimal use of land resources.

The goal of this study is to establish a diagnostic protocol for analyzing water transfer in soil, focusing on soil texture and gradients of salinity and sodicity in irrigation water. To achieve this, experiments will be performed using soil samples in Plexiglas columns under controlled conditions, where different salinity and sodicity gradients will be applied to various soil textures to simulate water movement. Control columns will receive irrigation with distilled water.

Preliminary results indicate that soil salinity and sodicity levels can be managed by adjusting irrigation water quality and considering soil texture, provided there is effective drainage. This strategy helps prevent physical soil degradation caused by sodicity. Therefore, it is important to select salt-tolerant crops for clayey soils with high sodicity and to implement early diagnostic measures to address the effects of salinity and sodicity, in order to maintain soil physical quality. has more than 40 % of Turkish sunflower production. Based on the study results, some candidate hybrids had higher drought tolerant indices such as total chlorophyll content, hairiness, dry root weight at stem than control hybrids in the study.

Keywords: water transfer, Salinity, Sodicity, Soil Texture, Irrigation, Soil Column.

INTRODUCTION

Soil and water salinity is one of the economically costly challenges for achieving sustainable development globally. Salinity poses a significant environmental issue, particularly in arid and semi-arid regions where precipitation is insufficient to leach salts from the root zone. Over 75 countries worldwide face salinity issues.

Recent trends and future demographic projections indicate that increasing food and fiber production will require effective use of salt-affected lands and saline water resources. Currently, at least 20% of irrigated lands globally are saline and/or irrigated with water containing high salt levels. Several major irrigated areas have experienced issues with salinity and sodicity, which have diminished their agricultural productivity and sustainability.

It is estimated that salinization of irrigated lands results in an annual global revenue loss of about $12 billion, impacting national economies in countries affected by saline land degradation and saline water resources.

Typically, the most severe impacts of salinity occur in areas where farming communities are relatively poor and face economic challenges. In extreme cases, salinization leads to the displacement of affected communities, with men seeking non-agricultural income opportunities elsewhere.

According to Gergely (2010), there are two processes of salinization: primary and secondary.

Primary salinization involves the accumulation of salts through natural processes due to high salt content in the parent material or groundwater. Secondary salinization is caused by human activities, such as inappropriate irrigation practices and inadequate drainage.

Saline soils account for approximately 40% of the global area affected by salt These soils are non-sodic and contain sufficient soluble salts to adversely affect the growth of most crops.
Sodic soils represent approximately 60% of the global area affected by salt. These are non-saline soils containing sufficient exchangeable Na^+ to impair agricultural production and soil structure under most soil and plant conditions.

Saline water is used to irrigate cultivated lands in various parts of the world, particularly in arid and semi-arid regions, which account for 41% of the Earth's surface (UNDP 1997), often with poor water quantity and quality. In these areas, limited precipitation (≤ 400 mm/year) is insufficient to leach salts from the root zone, leading to the accumulation of irrigation water salts in the soil and affecting its properties.

Saline water is not beneficial for irrigated agriculture, but it can still be a primary source of irrigation water in extensive arid regions, especially in developing countries where extreme freshwater scarcity and rapid population growth demand more water.
It impacts various physical and chemical properties of the soil, there by influencing its suitability as a growing environment for plants
The increase in soil salinity and/or sodicity affects soil structure, in turn, have significant impacts on soil hydraulic properties such as water retention capacity and hydraulic conductivity.
Soil hydraulic properties are among the most important and fundamental soil variables due to their effects on agricultural production, ecological and environmental protection, engineering, and construction. Hydraulic conductivity can be used to establish soil-water relationships.
Saturated hydraulic conductivity determines the soil's ability to transmit water and affects all soil-plant-water relationships and processes. It primarily depends on soil structure and texture. The hydraulic conductivity of soil can be altered by the composition or concentration of salts in the aqueous solution.
To address this challenge, it is crucial to gain a better understanding of the impacts of salinity and sodicity on soil hydraulic properties, as well as their interactions with other soil characteristics.

MATERIAL AND METHOD

In this study, we examined the saturated hydraulic conductivity of the soil using disturbed samples arranged in soil columns. The soil column is a classic laboratory tool widely used to study solute transport. We conducted several experiments with it, using different saline concentrations and soil textures. Soil texture is one of the most important characteristics of soil and is considered key to its fertility. It is related to many other soil properties and controls its physical, chemical, hydrological, and mechanical behavior. four soils with different textures were investigated in these experiments. The samples taken are first characterized in terms of their physicochemical properties. The disturbed soil samples were air-dried and passed through a 2 mm sieve. A portion of the sample was used for particle size analysis. Subsequently, soil texture classes were determined according to the USDA soil classification system (USDA, 1987).

To control both salinity and sodicity parameters, saline solutions were prepared using two salts: sodium chloride (NaCl) and calcium chloride ($CaCl_2$). The choice to combine these two salts is deliberate. On one hand, it allows for the expression of both beneficial and detrimental effects respectively attributed to Ca^{2+} and Na^{+} ions in the environment. On the other hand, it reflects the real-world scenario where a single salt, particularly NaCl, is rarely found in isolation, as salts generally occur in various forms or even complex combinations. We obtained four chosen values for Salinity concentration (0, 250, 450, and 775 méq/L) and four additional values for SAR (0 / 22,37 / 39,36 and 42,43), in accordance with the relationship between SAR and salinity concentration: The Sodium Adsorption Ratio (SAR) is a useful index for assessing soil sodicity or the relative sodium status of soil solutions. It is calculated as follows (Gregorich and Carter, 2007):

$$x = \frac{(\mathrm{Na+})}{\sqrt{(\mathrm{Ca2+}) + (\ \mathrm{Mg2+})/_{2}}}$$

We note that the range of sodicity (SAR) we have chosen includes a value considered average in the arid and semi-arid regions of Algeria, which is 15. In this context, the SAR value of 42,43 is intended to represent extremely severe aridity conditions in these regions.

Hydraulic Conductivity Measurement Setup

The setup for measuring saturated hydraulic conductivity (CHs) is based on the design described by Aubert (1978). It consists of four graduated columns connected to a tube, which is linked via a valve to a Woolff flask. This flask is fed from another Woolff flask using a valve. The upper Woolff flask provides water to the lower flask, which maintains a constant head on the sample columns. The lower flask supplies the columns through the tube. After equilibrium is reached between the solution and the sample, the volumes of the collected solutions are measured after one hour of percolation. During each irrigation, we make sure to keep a consistent stagnant water depth of 3 cm at the top of each soil column. Before measuring the CHs, we allowed the saline solution to percolate through the sample until the electrical conductivity (EC) of the collected drainage solution matched that of the saline solution. The equilibration time varies depending on the clay content and the relative levels of salinity and sodicity involved. The electrical conductivity (EC) is measured using a conductometer on a saturated paste extract. Distilled water, with zero salinity and sodicity, is used as the control solution. All CHs measurements are repeated three times.

RESULTS AND DISCUSSION

The soil infiltration process is indicated by changes in the infiltration rate, which reflect the soil's infiltration capacity. Variations in infiltration rates under four different salinity levels over time are presented in figure 1, 2 and 3. The graphs show an inverse correlation between the increasing salinity of the irrigation water and the hydraulic conductivity of the four textures, indicating a systematic reduction in the latter as salinity increases.
The results of saturated hydraulic conductivity as a function of salinity and sodicity and soil texture are presented in Figures 1, 2, and 3

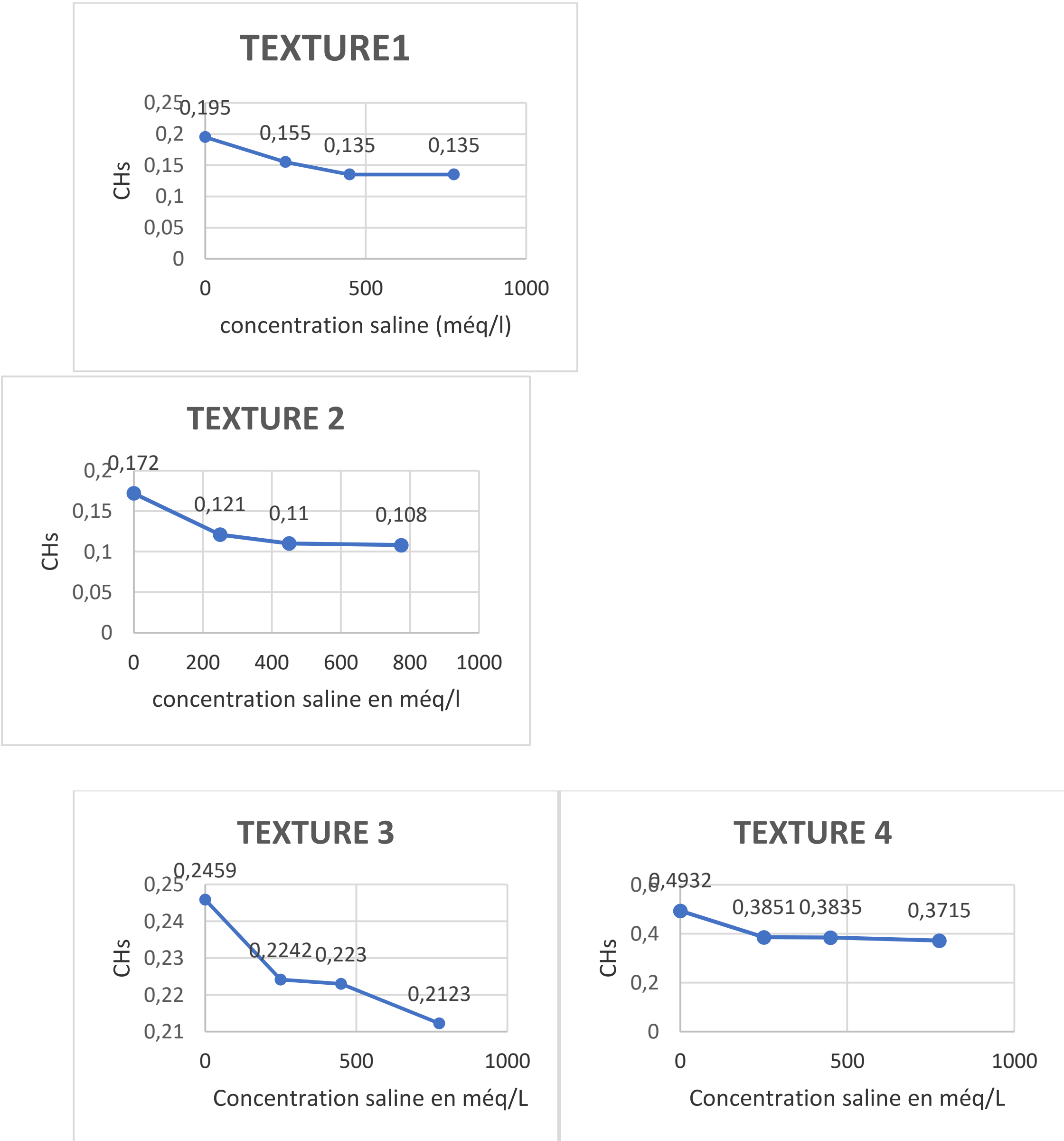


Figure 1: Soil hydraulic conductivity (Cm/min) affected by soil texture and salinity

Increased salinity in irrigation water results in higher concentrations of dissolved salts, which can alter soil physical properties. Elevated salinity can lead to the dispersion of soil particles, particularly in clay-rich soils (texture 2). This dispersion reduces soil aggregation and pore connectivity, thereby diminishing the soil's hydraulic conductivity.

Overall, the relationship between irrigation water salinity and hydraulic conductivity is characterized by a negative correlation, where higher salinity levels correspond to reduced hydraulic conductivity due to the detrimental impact of salts on soil structure and pore dynamics.

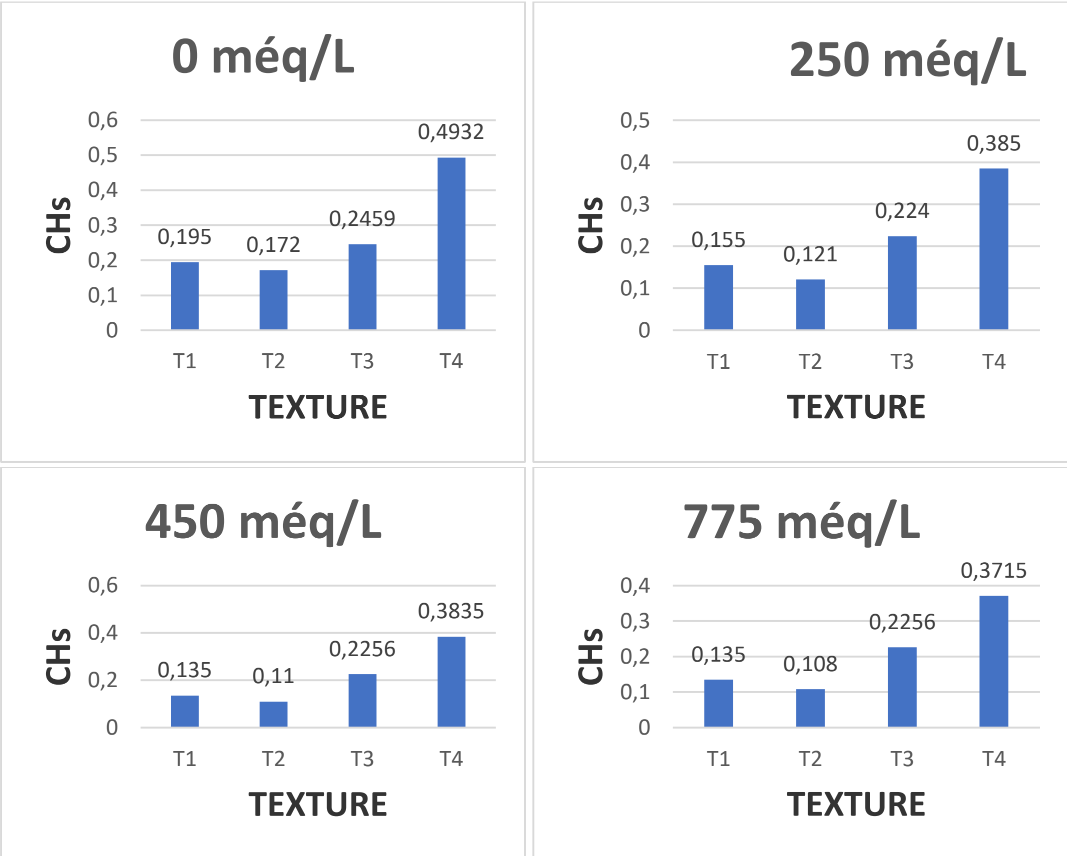


Figure 3: Soil hydraulic conductivity (Cm/min) affected by soil texture

The results indicate that the clay content percentage plays a crucial role in water movement through the soil. Soils with higher clay content tend to exhibit lower infiltration rates compared to soils with lower clay content.

This is likely due to the fact that clay particles, being fine and cohesive, reduce the soil's permeability and increase its capacity to retain water, thereby limiting the infiltration rate.

The impact of the salt content in irrigation water on water infiltration characteristics varies depending on the different soil textures.

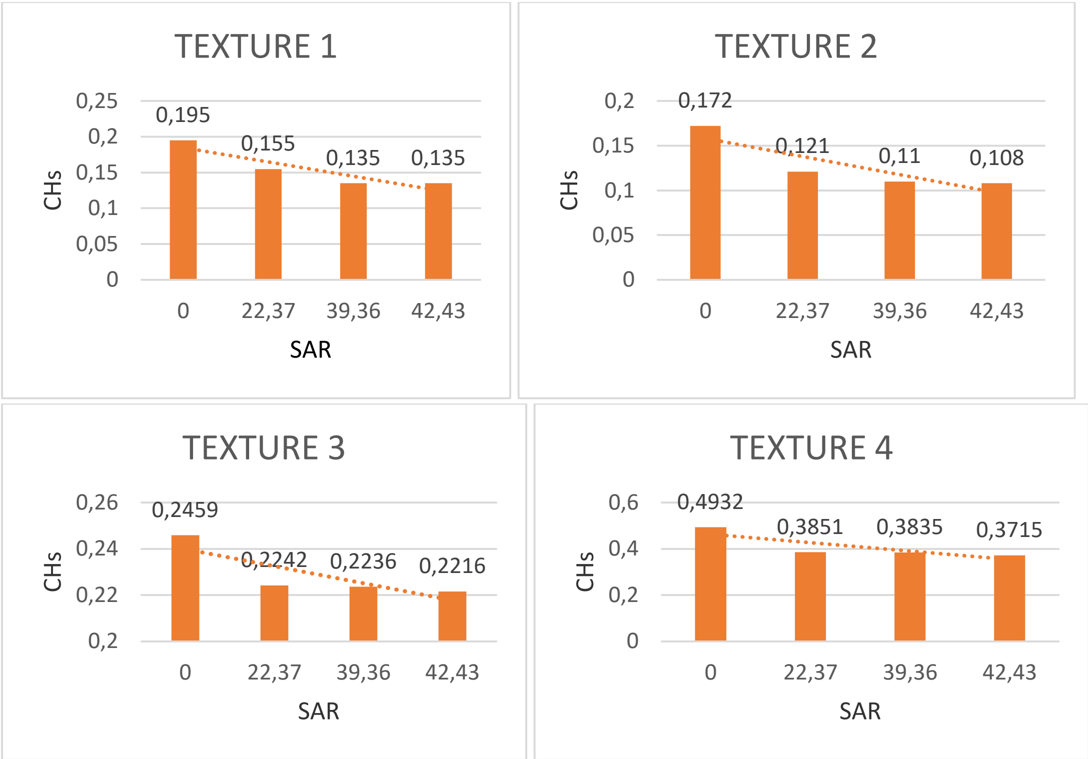


Figure 2: Soil hydraulic conductivity (Cm/min) affected by soil texture and sodicity

first general observation from these histograms is that sodicity tend to decrease the values of saturated hydraulic conductivity. The soil infiltration rate, decreased with increasing salt concentration in the irrigation water. The rise in sodium ions caused clay swelling upon wetting, which destroyed the soil structure by inducing swelling, settling, and dispersion of the clay. This led to the collapse of macropores and blockages, there by impeding water movement through the soil matrix.

CONCLUSIONS

This study was designed to provide detailed information on the effect of a wide range of saline and sodic solutions on the saturated hydraulic conductivity of several well-characterized soils.

Irrigation with water having a high SAR leads to an increase in the exchangeable sodium percentage (ESP) in the soil, which negatively impacts its physical properties, such as hydraulic conductivity (HC), due to clay dispersion caused by Na+ ions. Changes in soil sodium content can also lead to alterations in the physical and chemical properties of the soil, affecting the composition of cation exchange complexes and mineral composition. This, in turn, influences how soil particles interact and bond with each other within aggregates.

However, the effects of sodium and salt concentrations on soil hydraulic properties were also influenced by other soil properties, such as soil texture.

Saturated hydraulic conductivity is a key determinant of a soil's ability to transmit water and plays a critical role in the interactions and processes among soil, plants, and water. This property is mainly influenced by the soil's structure and texture. Furthermore, the hydraulic conductivity of the soil can be affected by the concentration and composition of salts in the aqueous solution, which can modify the soil's hydrodynamic behavior.
Studying the effects of soil salinity on hydraulic conductivity will provide valuable insights for developing irrigation systems and implementing salt leaching methods.

REFERENCES

Bouwer, Herman. « Integrated Water Management for the 21st Century: Problems and Solutions ». *Journal of Irrigation and Drainage Engineering* 128, nº 4 (1 août 2002): 193-202. https://doi.org/10.1061/(ASCE)0733-9437(2002)128:4(193).

Ghassemi, Fereidoun, A. J. Jakeman, et H. A. Nix. *Salinisation of Land and Water Resources: Human Causes, Extent, Management and Case Studies*. CAB International, 1995.

Halitim, Amor. « Contribution à l'étude des sols des zones arides (hautes plaines steppiques de l'Algerie): morphologie, distribution et rôle des sels dans la genèse et le comportement des sols ». PhD Thesis, Ecole Nationale Supérieure Agronomique, 1985.

Latef, A. Abdel. « Changes of Antioxidative Enzymes in Salinity Tolerance among Different Wheat Cultivars ».
Cereal Research Communications 38, nº 1 (11 mars 2010): 43-55. https://doi.org/10.1556/crc.38.2010.1.5.

Minhas, P. S. « Saline water management for irrigation in India ». *Agricultural Water Management* 30, nº 1 (1 mars 1996): 1-24. https://doi.org/10.1016/0378-3774(95)01211-7.

Pitman, Michael G., et André Läuchli. « Global Impact of Salinity and Agricultural Ecosystems ». In *Salinity: Environment - Plants - Molecules*, édité par André Läuchli et Ulrich Lüttge, 3-20. Dordrecht: Springer Netherlands, 2002. https://doi.org/10.1007/0-306-48155-3_1.

Qadir, M., B. R. Sharma, A. Bruggeman, R. Choukr-Allah, et F. Karajeh. « Non-conventional water resources and opportunities for water augmentation to achieve food security in water scarce countries ». *Agricultural Water Management* 87, nº 1 (10 janvier 2007): 2-22. https://doi.org/10.1016/j.agwat.2006.03.018.

Qadir, M., A. Tubeileh, J. Akhtar, A. Larbi, P. S. Minhas, et M. A. Khan. « Productivity Enhancement of SaltAffected Environments through Crop Diversification ». *Land Degradation & Development* 19, nº 4 (juillet 2008): 429-53. https://doi.org/10.1002/ldr.853.

Rengasamy, P., et K. A. Olsson. « Sodicity and Soil Structure ». *Soil Research* 29, nº 6 (1991): 935-52. https://doi.org/10.1071/sr9910935.

She, Xuemei Gao, Peng Wang, Wentao Xu, Yingying Liu, et Yi Liu. « Comparison of soil hydraulic properties with different levels of soil salinity and sodicity | Arabian Journal of Geosciences », 2014. https://link.springer.com/article/10.1007/s12517-014-1514-8.

So, H. B., et L. a. G. Aylmore. « How Do Sodic Soils Behave - the Effects of Sodicity on Soil Physical Behavior ». *Soil Research* 31, nº 6 (1993): 761-77. https://doi.org/10.1071/sr9930761.

Wichelns, Dennis, et J. D. Oster. « Sustainable irrigation is necessary and achievable, but direct costs and environmental impacts can be substantial ». *Agricultural Water Management*, Responsible Management of Water in Agriculture, 86, nº 1 (16 novembre 2006): 114-27. https://doi.org/10.1016/j.agwat.2006.07.014.

APPLICATION OF EMULSION BASED ENCAPSULATION METHODS IN FOOD TECHNOLOGY: A REVIEW

Jasmine Tahmaz

Faculty of Agriculture and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

ABSTRACT

Encapsulation is a method of entrapping and protecting sensitive active compounds into the structure of an encapsulation carrier (coating material). Capsules of different dimensions (macro, micro, and nano) are obtained as encapsulation products. Each capsule consists of two main materials: 1) active compound and 2) carrier (coating material). The main aim of encapsulation is to protect active compounds from degradation influenced by different external factors. Encapsulation is a technique that can increase the stability and improve the usability of many active and biologically valuable natural ingredients. In recent times it is increasingly used in food technology. Emulsification is one of the most important encapsulation methods suitable for use in food technology. It can be used alone or in combination with other encapsulation methods. Formation of emulsion can be used for the encapsulation of hydrosoluble and liposoluble liquid substances. The size of droplets within such emulsions ranges from 0.1-5000 μm. The main advantages of this encapsulation method are good protection of the encapsulated substance from high temperatures and oxidation during heat treatment and drying, the possibility of encapsulating liposoluble and hydrosoluble substances, and controlled release of the active substance. Emulsion-based techniques are widespread encapsulation methods suitable for the food industry. A wide range of active substances can be encapsulated, such as probiotic bacteria, proteins, amino acids, essential oils, flavonoids, vitamin E, lutein, beta carotene, fish oil, omega 3 fatty acids, aspartame and other sweeteners, xylitol and menthol in chewing gum (prolonged cooling effect), curcumin, catechin, vitamin C, vitamin B12 (for the enrichment of dairy products), vitamin B1 and herbal extracts. Obtained capsules can be applied in the production of functional milk and dairy products, salad sauces and dressings, fruit juices, dried soup mixtures, functional meat products, the oil industry, and confectionery.

Keywords: Encapsulation, Emulsions, Food technology, Stability, Enrichment

INTRODUCTION

Encapsulation is a technique by which a material or mixture of materials is packed into the structure of another material. The beginnings of encapsulation use in industry date back to around 1950th years (Madene et al., 2006). Firstly it was commonly used in the pharmaceutical industry and in in recent time in food industry, too. For the needs of the food products, the most common encapsulated active compounds are: vitamins, minerals, dyes, enzymes, probiotics and flavors.

The importance of encapsulation lay in fact that it is a technique that can improve the stability, usability and availability of many biologically valuable natural ingredients. The goal of encapsulation is the formation of stable capsules from natural or synthetic polymer materials within which the active substance is encapsulated (Pegg and Shahidi, 1999).

Each capsule consists of two basic compounds: 1) coating material (carrier, sheath, wall material, shell or encapsulation matrix) and 2) active substance (encapsulant, inner phase or capsule core).

The active substance is the material that is encapsulated inside the capsule. The coating is the material which coats and protect active compound in its structure. The role of the carrier is to create a boundary layer between the active substance and the environment. The choice of carrier depends on the size and shape of the capsule, the cost of encapsulation, encapsulation method, and the mechanism of release of the active substance. Edible and harmless biopolymer substances, which do not react with the active substance, are used as carriers.

The most common reasons for applying encapsulation are:

1. Protection of the active substance from external influences, i.e. from degradation under the influence of external factors (heat, oxidation, moisture, air, light, chemical ingredients with which it can react).

2. Enrichment of food products with biologically valuable active ingredients (vitamins, minerals, omega fatty acids), which are often sensitive and easily decomposed.

3. Modification of the physical characteristics and structure of the original material, which makes it possible to achieve easier handling, improvement of the texture of the product into which they are dosed, and better rheological properties. E.g. liquid substances can be inserted into solid materials by encapsulation. It is possible to prevent the formation of lumps, reduce hygroscopicity, improve flowability, and modify the consistency of the medium to which the capsules are added.

4. Controlled release of the active substance (gradual, slow or at the exact desired moment). The release of the active substance in the product is slower if the substance is encapsulated. E.g. by encapsulating the sweetener, a gradual release of the sweet taste is achieved.

5. Uniform distribution of the active substance when added to different food products.

6. Encapsulation can dilute the concentration of the active substance, which is significant when it is necessary to uniformly mix very low concentrations of the active substance in a larger amount of product.

7. Masking of unwanted taste, smell and aroma (e.g. encapsulated fish oil, bitter substances, etc.)

8. Prevention of unwanted reactions of the active component with the ingredients inside the product.

ENCAPSULAION METHODS AND CAPSULE TYPES

Encapsulation methods can be devided into two main groups: mechanical and chemical. In food technology, the most common encapsulation methods are: spray drying, emulsion based methods, centrifugal separation, coacervation, liposomal capsules and fluidization (fluid bed coating).

Capsule types

The basic types of capsules are illustrated in Figure 1. Considering the structure and the way the carrier is arranged around the active substance, there are three basic types of capsules (Zuidam and Shimoni 2010):

1. Aggregate, matrix or microsphere type – the aggregate structure of the capsule. These are capsules that have several separate cores of the active substance or, more often, a larger number of central particles (molecules or groups of molecules of the active substance that make up the particle) embedded within a continuously distributed matrix or carrier. This is the most common type of capsule and is obtained by applying the largest number of encapsulation techniques. Encapsulant molecules can also be found on the surface layers of the capsule. Therefore, this is the least efficient way of encapsulation.

2. Type of central accumulation. It is the simplest and most obvious form of capsule structure. The core with the encapsulated content is spherical in shape, surrounded by a shell or membrane of uniform thickness like a hen's egg and shell. The thickness of the coating and the proportion of the active substance can be different (Pegg and Shahidi, 1999).

3. Type of multilayer casing. The sheath is wrapped around the core in the form of two or more concentric layers of different materials. Such capsules are designed to allow controlled release of the active substance (Pegg and Shahidi, 1999).

Capsules are not always spherical or other regular shape. The active substance can be evenly or unevenly distributed inside the capsule (Bakry et al. 2014). Also, there are cases where already formed smaller capsules are encapsulated inside larger capsules (capsule within a capsule). The reason for this kind of encapsulation is when one wants to achieve the intended controlled release of the active substance. Therefore, these three basic types of capsules can differ in shape, structure and size. In addition, depending on the encapsulation carrier and the method of formation, capsules can have a different internal structure (Rodriguez et al. 2016).

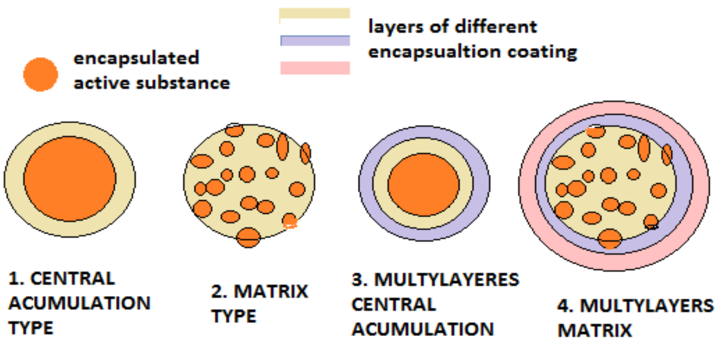


Figure 1 Types of capsules

A matrix-type capsule (with multiple separate encapsulated regions) is often referred to as a microsphere. Liposome capsules are capsules of the central accumulation type where the encapsulation carrier completely covers the encapsulated substance. In the case of liposomal capsules, the carrier (coating) is formed from a double layer of phospholipid molecules that cover the active substance in the form of a film. The phospholipid bilayer in the liposomal capsule is formed by connecting the non-polar ends of phospholipid molecules to each other, forming a spherical particle (liposome), while the polar groups remain oriented towards the surface and inside the particle. A cyclodextrin capsule is a chemical complex in which the active substance is encapsulated in the empty inner part of the ring of the cyclodextrin molecule. In this way, the active substance forms a chemical complex with the cyclodextrin molecule, which is why this method of encapsulation is called molecular inclusion. Capsules in emulsion actually represent particles of an active substance coated on all sides with particles of a surface-active substance (emulsifier or stabilizer). In this way, a hydrophobic active substance is stabilized in a hydrophilic environment or vice versa (Rodriguez et al. 2016).

Micelles are complex particles formed by aggregation or self-association of the same or similar molecules (most often proteins such as casein). Aggregation and self-association of molecules in the micellar structure is achieved by hydrophobic and/or electrostatic interactions. The main feature of the micelle is the hydrophilic character on the surface and the hydrophobic interior. Under the influence of physical factors, micelles can dissociate into their constituent parts and reassociate again, i.e. to associate. Precisely because of this, the encapsulation of smaller molecules within the micellar structure is possible. Casein micelles are the most common coating material in the formation of micellar type capsules. Different active compounds with particle size smaller then micelle could be encapsulated inside micellar structure of casein or other proteins. The structure of casein micelle allows encapsulation of small molecules of active compounds and formation of matrix type capsules. Casein micelle is the spherical particle with common size 100-200 nm and consisted of smaller spherical particles (submicelles) bound together in micellar structure. Submicelles are bounded together in the micellar structure through pretty strong colloidal calcium phosphate bridges. Beta carotene can be encapsulated inside the micellar casen capsules. Such kind of encapsulation can be improved by high hydrostatic pressure induced dissociation and reassociation of casein micelle. Dissociation of micelle helps incorporation/encapsulation of beta carotene inside micellar structure, Reassociation of casein micelle with encapsulated beta carotene occurs when pressure decreased (Tahmaz 2014).

EMULSIONS AND THEIR PREPARATIONS

Emulsification is one of the most common methods in encapsulation (immediately after spray drying), whether it is applied alone or as part of other encapsulation methods.

An emulsion is a stable mixture of two liquids between which high surface tension occurs and which therefore cannot mix. A large surface tension in between occurs as a result of the difference in polarity. Polar and non-polar liquids do not mix together. Typical examples of polar liquids are water, salt and electrolyte solutions, polar organic liquids (alcohols, acids, etc.). Emulsion is obtained by vigorous mixing of polar and non-polar liquid. Vigorous mixing breaks up the layers and breaks up their droplets. In this way, the total contact surface between the particles of polar and non-polar liquid increase, and their merging into a mixture occurs. The emulsion created by vigorous mixing is unstable, because the particles of polar and non-polar liquids do not show affinity towards each other. Although they are mixed, there is still a high surface tension between them, which is why they repel each other and delamination occurs very quickly. On the other hand, mutual attractive forces between polar and non-polar liquid molecules are activated. As a result of these attractive forces, polar particles are regrouped on the one side, and non-polar on the other and emulsion separate to two phases. In other words, vigorous mixing without the presence of a surface-active substance will result in formation of unstable emulsion. Such emulsion is not thermodynamically stable, but is only kinetically stable for a short time.

Every stable emulsion consists of three basic parts: a polar (hydrophilic, lipophobic) liquid, a non-polar (hydrophobic, lipophilic) liquid and a surfactant. In order to obtain a thermodynamically stable emulsion, it is necessary to reduce the surface tension between polar and non-polar liquids so that they remain mixed. Emulsifiers are surface-active substances, whose molecular structure is such that they have polar (charged) and non-polar (non-charged) parts in the same molecule. Polar moieties are mostly functional groups with oxygen such as OH- group or some other groups that have free electrons. Nonpolar groups are nonpolar ends (radicals) of organic molecules (eg fatty acid radicals). Typical examples of emulsifiers are lecithin, phospholipids, fatty acid monoesters, milk proteins, other proteins, etc. When an emulsifier is added to an unstable emulsion, the polar end of the emulsifier connects to the polar liquid, while the nonpolar end connects to the nonpolar liquid.

In the food industry, the emulsification process is often used to obtain stable food products. A large number of food products are typical emulsions. Typical examples of natural food emulsions are milk, butter and egg yolks. In milk, the milk fat is emulsified in the aqueous phase with lecithin and milk proteins as emulsifiers. Typical food emulsions obtained by artificial emulsification (in the production process) are mayonnaise, margarine, cream, sour cream, cake mixes, baby food, etc.

Emulsions can be divided into different types depending of amount and nature of each phase (Figure 2):

1. Single emulsions:
   a. Oil-in-water emulsions - known as o/w emulsions (eng. oil/water)
   b. Water-in-oil emulsions - known as w/o emulsions (eng. water/oil)
2. Double emulsions:
   a. Oil in water and all in oil - o/w/o (oil/water/oil)
   b. Water in oil and all in water - w/o/w (water/oil/water).
3. Multiple emulsions (eg. o/w/o/w or w/o/w/o)

Oil-in-water (o/w) emulsions have more intense properties of the water polar phase. They can be mixed with water or other polar liquid, show higher values of electrical conductivity (due to higher water content and higher polarity). Components that are soluble in water can be mixed in them. Emulsifiers soluble in water are added to this type of emulsion to stabilize them. A typical example of such emulsions is milk and cream. In water-in-oil (w/o) emulsions, the dominant phase is oil (non-polar phase). These emulsions are soluble in oils, have low electrical conductivity (due to the low content of polar components). Typical examples of w/o emulsions are butter and margarine.

Some products show properties of both types of emulsions (w/o and o/w) at the same time. The characteristic of these emulsions is that they can be mixed with both oil and water phase, which means that liposoluble and hydrosoluble active components can be added to them. They are considered stable emulsions, because they show good affinity towards polar and non-polar substances. Examples of such emulsions are mayonnaise, cake and pancake mixes and many other food products. Although it contains greater amount of oil phase mayonnaise is defined oil in water emulsion. These are mixed emulsions, which are very stable, because they contain a relatively high proportion of emulsifiers and/or an equal proportion of polar and non-polar phases. This is exactly why they have the properties of o/w and w/o emulsions.

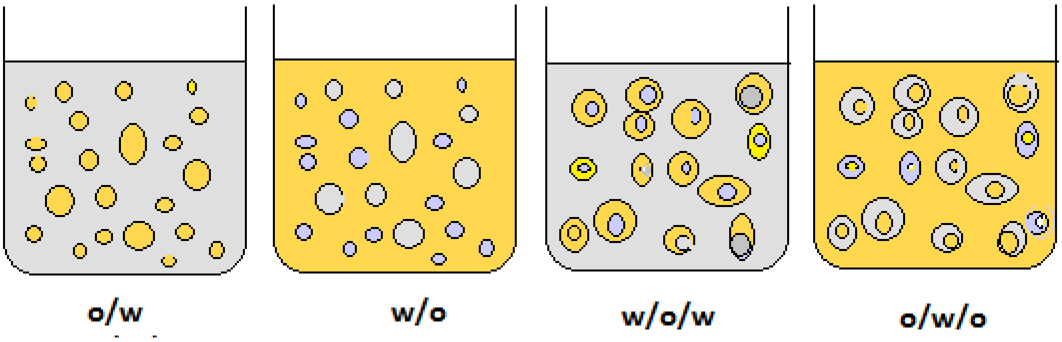


Figure 2 Illustrations of emulsion types

Multiple emulsions (Figure 2) can be double (w/o/w and o/w/o) or even triple (w/o/w/o and o/w/o/w). These emulsions are obtained by the process of double or multiple emulsifying. They can be considered as an emulsion dissolved in an emulsion. Preparation of double or triple emulsions includes the following steps. The first step is preparation of stable primary w/o or o/w type emulsion. The primary emulsion is dosed into another liquid, in which it could not be mixed into stable mixture without emulsifying. But, in the presence of emulsifier or other surface-active substance, mixing occurs and a multiple emulsion is formed in which the droplets of the original emulsion are dispersed and stabilized. For example, in the case of a w/o/w emulsion, a stable water-in-oil emulsion is first formed. Water in oil emulsion mostly shows properties of oil phase and it cannot be mixed with water phase in stable mixture without emulsifying. Because of that primary water in oil emulsion is added to the polar water phase with vigorous mixing in the presence of emulsifier. Vigorous mixing help to primary emulsion to be mixed en dispersed in water phase, while presence of an emulsifier ménages to stabilize double emulsion. In an o/w/o emulsion, an oil-in-water type emulsion is firstly formed, which is then added to the non-polar fat phase with which it is mixed and stabilized in the presence of a suitable emulsifier. These procedures of additional emulsification can be repeated several times according to the same principle, so that quadruple emulsions and other more complex emulsions can be obtained.

The key factor for emulsion stability is homogenization, i.e. the better dispersion of particles of the dispersing phase within the dispersing agent (continuous phase). Therefore, it is necessary that the particles of the dispersed phase be as small as possible. Thus, a larger specific surface area of the continuous phase particles and a larger contact surface between the dispersed phase and the dispersing agent are achieved. The smaller the particles, the more stable the emulsion. Emulsion homogenization can be done with different devices:

1. High speed vertical mixers – the mixing speed is not high and they usually give weak emulsions whose particle sizes are around 10 µm

2. Homogenizers – devices that operate on the principle of passing liquid material through small holes under high pressure

3. ultrasonic mixers/homogenizers – for homogenization, that use ultrasonic energy, pulverize dispersed phase particles to very small dimensions (nanoparticles), produce very stable emulsions (nanoemulsions)

4. colloid mills – which have fast rotation, where they grind particles to colloidal dimensions

5. membrane homogenizers where the particles of the dispersed phase are passed through the microporous membrane and

6. microfluidizers.

With the membrane process, it is possible to obtain fine and stable emulsions. This is why the encapsulation process itself is very successful, because very small and stable capsules are formed in such emulsions. Emulsifying using a membrane homogenizer has proven to be a very successful method for encapsulation. It is a process that involves passing the pre-emulsion through a microporous membrane. Depending on the size and character of the pores (hydrophilic or hydrophobic pores), it is possible to encapsulate hydrophobic and hydrophilic active substances. Hydrophilic active substance is encapsulated in w/o, and hydrophobic in o/w emulsion.

ENCAPSULATION BY EMULSIFICATION

After spray drying, emulsions and emulsifying processes are the second most common encapsulation technique used in industry. The commercial possibilities of using double emulsions are reflected in the production of low-calorie foods (sauces, mayonnaise, whipped cream, etc.) and for the encapsulation of nutritionally valuable ingredients, flavors and additives (Muschiolik and Dickenson 2017). Some examples of encapsulation using the double emulsification technique are given in Table 1. Emulsifying can be used as primary and main encapsulation method or it can be combined with other encapsulation methods like spray drying, freeze drying, high hydrostatic pressure to obtain more stable particles.

Emulsification can be used to encapsulate water-soluble and lipid-soluble liquid substances. The size of the droplets inside such emulsions ranges from 0.1-5000 μm. The stability of the encapsulated substance can be improved by forming a multilayer shell (Prichapan and Klinkesorn 2014). Encapsulation proceeds by first dissolving the encapsulation carrier in an organic solvent (non-polar - organic phase), and the active substance is added to that solution. An aqueous solution containing an emulsifier is added to the organic phase. By mixing these solutions, an emulsion is formed. The organic solvent is removed by evaporation, while the carrier polymer molecules form a shell around the active substance. The obtained emulsion can be spry or freeze dried (Silva et al 2014). Encapsulation in different emulsion types and images of obtained capsules are illustrated in Figure 3,

Encapsulation by emulsification can be carried out in single or double emulsions. During emulsion base encapsulation, two basic processes are crucial: 1) the emulsification of the active substance in the carrier solution and 2) the precipitation of the obtained capsules (Figure 3).

Encapsulation efficiency is increased with the addition of cross-linking agents. The formation of cross-links can be assisted physically (precipitation of proteins by heating) or chemically (precipitation after the addition of cations - usually Ca2+ ions or by lowering the pH value). The encapsulation carrier is dissolved in an aqueous medium containing a cross-linking agent (CaCl2). The resulting solution is mixed with oil or another hydrophobic medium in which the active substance is dissolved. Smaller particles of the active substance remain encapsulated within the cross-linked structure of the encapsulation carrier (Mannyana et al., 2010).

Coaxial mrofluidizer can be used as a device, which is able to form very small microdroplets in emulsions for encapsulation. Using this device, microcapsules of the central accumulation type are obtained in single, double and triple emulsion. The advantage of using a coaxial encapsulator is its possibility to form capsules of uniform and regular spherical shape and size with a thin shell with uniform thickness (Li et al. 2018). This method is very similar to encapsulation by co-extrusion, because the active substance is coated when leaving the microfluidizer in the same way as in co-extrusion.

Pickering emulsion is emulsion stabilized by fine solid particle, which absorb irreversibly on emulsion droplets. It is very applicable for encapsulation in functional food products (Cheon et al., 2023).

Capsules of different types can be formed by encapsulation in an emulsion (FIGURE 68), which depends on the emulsification method, the type of emulsifier, stabilizer and biopolymer, the aggregate state of fat droplets and the water phase. In an ordinary single emulsion, the active substance is stabilized only by a layer of emulsifier molecules. In the case of double emulsions, a single emulsion is first formed, which is then dissolved in an aqueous or fat phase different from the dispersing agent of the primary emulsion. If the aqueous phase contains a biopolymer that causes gelation (gelatin, pectin, starch, alginate), then an emulsion in the gel state is obtained. In such an emulsion, the fat droplet with the active component is coated with emulsifier molecules, and around it is a gelled water phase. Gelation further stabilizes the emulsion. Encapsulation in an emulsion can be achieved by coating the active component with solid colloidal stabilizer particles. If the oil phase has a high melting point, then by emulsification it is possible to obtain capsules of the SLP type, i.e. solid lipid particles coated with emulsifier molecules. For the formation of such emulsions, heating is necessary in order to melt the fatty phase. The dissolved fat phase is emulsified in an aqueous solvent, the small droplets are coated with emulsifier molecules, and after cooling, the encapsulated fat droplets return to a solid aggregate state (Mao and Miao 2015).

Encapsulation in single emulsions

If the active substance is lipophilic (eg. beta carotene), it can be encapsulated in o/w emulsions. Encapsulation occurs when a hydrophilic shell of surfactant and carrier is formed around the beta-carotene particle. The hydrophilic coating enables the solubility of beta-carotene in the aqueous environment (Ax 2003; Ribeiro et al. 2010). If the active substance is hydrophilic, then the encapsulation is carried out in a w/o emulsion, and the surface of such a capsule is lipophilic (Manyanna et al. 2010). Shematic representation of formation of capsules in single emulsion is showed in Figure 3.

Peng et al. (2023) investigated influence of emulsion particle size on encapsulation efficiency of encapsulated sweet orange essential oil into maltodextrin and modified starch capsules. Emulsion was prepared by mixing of dispersed phase containing orange oil with continuous phase containing coating material dissolved in water. After completed homogenization emulsion was divided into three phase with large, small and nano sized capsules. The results showed that encapsulation efficiency increased when capsule size decreased.

Fitri et al. (2022) reported very high values of encapsulation efficiency (96.6-99.88%) of beta carotene encapsulated in nanocellulose by preparing of o/w single emulsion. Zeta potential of obtained particles ranged between -8.69 and -19.73 mV.

Tessaro et al. (2022) encapsulated polyphenol rich pitanga leaf extract in soybean oil using single w/o emulsion. The obtained particles had sizes between 250 and 4250 µm and improved stability of antioxidative compounds. Extract firstly was dissolved in aqueous phase. Water in oil (w/o) emulsion was prepared by dropwise addition of aqueous extract into soybean oil containing lipophilic emulsifier PGPR under continuous high speed ultrasound homogenization (15000 rpm for 5 minutes). Emulsion droplet size ranged between 0.25 and 4250 µm depending of emulsifier and extract concentration. Emulsion wit 20% of extract concentration had the smallest particles and the highest physical stability.

Table 11. Application of double emulsion encapsulated active compounds in different food industrie (Muschiolik and Dickenson 2017)

Emulsion type	Active compound	Application in foods
w/o/w and o/w/o	Flavours encapsulated in gelatinizing polysaccharides	Addition in preparation of low calorie salad dressings, margarine and similar spreads
w/o/w	Vitamins encapsulated in 40% sucrose solution	Enrichment of confectionery products
w/o/w	Polyunsaturated seed oils	In different products that commonly contain milk fat, replacement of milk fat with encapsulated oils
w/o/w	w/o emulsin droplets	Replacement of oil or fat in low-calorie products (e.g. meat dough for the production of sausages and pate), drops have a lower oil content than oil alone, but give the perception of taste, aroma and texture as oil alone, improved sensory properties of low-calorie products
w/o/w	Na-ascorbate	Enrichment of milk before UHT sterilisation. Improved stability of ascorbate under thermal ttreatment
w/o/w	$CaCl_2$	Fortification of soy milk with Ca without of changes in consistency of soy milk. Encapsulated Ca does not change the consistency of milk, unlike non-encapsulated, which can lead to curdling
w/o/w	Natural colors	Application in confectionery industry and ice cream production – coloring of different products. Natural colors (beta carotene, anhocyanins) are

		mostly antioxidative compounds, very sensitive for oxidation and high temperature. Becaus eof that these colors are more stable in encapsulated form
o/w/o	Fish oil	Fortification of margarine and other spreads
w/o/w	Polyunsaturated oils	Replacement of pig fat with encapsulated oils in frakfurter sausages.
w/o/w	Sunflower oil	Fortification of milk
w/o/w	Vitamin B_{12}	Application in technology of functional dairy products
w/o/w	Aspatrame and other sweetners	Application in production of chewing gums. Encapsulated sweetners are released slowly then non encapsulated, and becaus eof that sweet taste is released gradualy.
w/o/w	xylitol and menthol	In the production of chewing gum - encapsulation achieves a prolonged cooling effect and keeps the menthol aroma longer
w/o/w	$MgCl_2$	As a coagulant in the production of soy milk cheese - encapsulation masks the bitter taste
w/o/w	Curcumin and catehine	Fortification and colouring of different drinks, juices and beverages
w/o/w	Grape seeds extract and saffron extract	Enrichment of different food products
w/o/w	NaCl	Application in production of low salt products. Increased perception of salty taste in products with reduced NaCl content, destabilization of the double emulsion with encapsulated NaCl occurs in the mouth, NaCl is gradually released.
w/o/w	Probiotics	Fortification of different foods
w/o/w and o/w/o	Flavours encapsulated in gelatinizing polysaccharides	Addition in preparation of low calorie salad dressings, margarine and similar spreads

Encapsulation in double emulsions

Encapsulation using the double emulsification technique is more often used, because smaller capsules can be obtained in comparison to single emulsion. The formation of capsules in double emulsions is illustrated in Figure 3. W/o/w emulsions are more often used for encapsulation needs in food technology in comparison to o/w/o emulsions. Some examples of application of double emulsion in encapsulation for purpose of food products are shown in Table 1.

In the case of lipophilic active substance, encapsulation can be achieved in an o/w/o emulsion, and in that case the encapsulation carrier is dissolved together with the emulsifier in the aqueous phase. The two phases are vigorously mixed to form a primary emulsion, which is allowed to stand for a period of time before a new amount of the aqueous phase will be added. By adding an aqueous phase, a double (secondary) emulsion of the o/w/o type is formed in which biopolymer particles with an encapsulated substance are deposited. The re-dosed aqueous phase destabilizes the primary emulsion (o/w), causing the capsules to separate. During this process, interactions occur between molecules of the encapsulation carrier, which leads to the formation of cross-links. This process contributes to the encapsulation, and the finer particles of the active substance stabilized in the oil remain encapsulated between the cross-linked carrier molecules (Manyanna et al 2010). Double o/w/o emulsions are mainly used for encapsulating different oils. Especially it is interesting to encapsulate fish oil in double o/w/o emulsion with purpose to mask its smell. Encapsulating fish oil masks its unpleasant odor and aroma, so that it can be added to different food products. In this emulsion type, fish oil is dispersed in water phase, while the secondary oil phase differs from the primary one (o1/w/o2). The most common emulsifier in the primary emulsion (o1/w) is gluten, while in the secondary emulsion it is Na-caseinate. In this type of emulsion, gluten and Na-caseinate are not only surfactants, but also encapsulation carriers. The encapsulation of fish oil via a double o1/w/o2 emulsion takes place in the following order. In the first step, fish oil is dispersed in an aqueous phase containing Na-caseinate, forming a primary emulsion. The primary emulsion is mixed with a second oil phase consisting of equal parts of olive oil and polyglycerol-polyricinoleate (surfactant) to form a secondary emulsion. Capsules are isolated/separated by spray drying. Before drying, the double emulsion is mixed with an aqueous phase with disolved Na-caseinate and lactose monohydrate dissolved (Muschiolik and Dickenson 2017).

Double w/o/w emulsions are used for encapsulation of hydrophilic active substances such as anthocyanins, phytosterols, betalains, natural colors, probiotics, B group vitamins, vitamin C, mineral salts (NaCl, CaCl2, MgCl2) and sweeteners. Lecithin is the most often used as an emulsifier, while gelatin, Na-caseinate and whey protein isolates are used as carriers coating materials). In this type of emulsion, the secondary emulsion water phase can be the same as in the primary (w/o/w), or it can be different (w1/o/w2). The primary emulsion usually contains lecithin, which stabilizes the hydrophobic active substance, while the second aqueous phase w2 represents a solution of a surface-active encapsulation carrier. Encapsulation occurs only after the addition of the second aqueous phase (w2) with another biopolymer, whereby biopolymer 2 simultaneously stabilizes the new secondary emulsion and forms a shell around the droplets of the primary emulsion containing the particles of the active substance. The first water phase often contains NaCl, CaCl2 or glucose in its composition. These components help form the coating on the surface of the capsule. NaCl and glucose increase the osmotic pressure, which leads to the precipitation of biopolymers, while Ca2+ ions cause aggregation by forming cross-links between carrier molecules and thus help the precipitation of capsules (Muschiolik and Dickenson 2017).

The encapsulation of fish oil in a two-layer (secondary) emulsion is carried out to prevent oxidation of omega 3 fatty acids and to mask the unpleasant smell of fish oil. The primary emulsion is formed by homogenizing fish oil and the aqueous phase with lecithin at pH 3. First, a negatively charged layer (lecithin) is formed on the surface, and the secondary emulsion can be obtained by adding a cationic stabilizer (chitosan). The anionic layer of lecithin binds chitosan cations and a positively charged particle coated with a lecithin-chitosan layer is formed (McClements et al. 2007).

Dang et al. (2024) encapsulated acerola juice (rich in vitamin C) with maltodextrin and gum arabica as coating materials by preparing of double w/o/w emulsion. Firstly the primary w/o emulsion was prepared by mixing of acerola juice with oil in proportion 40:60 and with addition of lipophilic emulsifier PGPR. The mixture of juice, oil and emulsifier was virgously stirred by mixer speed of 700 rpm. When primary w/o emulsion was prepared and stabilized, the secondary water plus emulsion was finished maltodextrin and gum Arabica dissolved in water phase was added. Water phase with dissolved wall material and emulsifier (Tween 20) was added slowly drop by drop into primary emulsion with continuous high speed mixing (900 rpm). Encapsulation yield of vitamin C ranged between 20.83 and 28.36%.

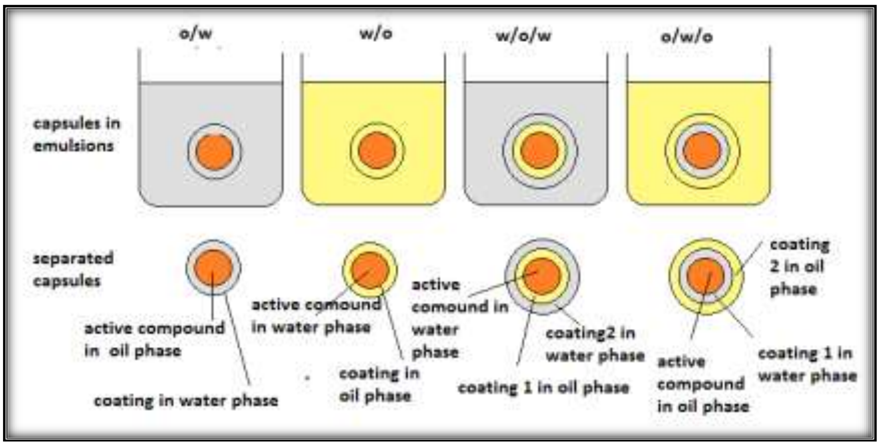


Figure 3 Illustration of capsules formed in emulsions

Encapsulation In multilayered emulsions

The formation of a multilayer shell around the active substance in the emulsion significantly increases the stability of the encapsulated active substance. Multyleyerd emulsions are known as m-o/w (multilayered o/w emulsions) or m-w/o (multy layered o/w emulsions).

Capsules with a multilayer shell produced most often in oil-in-water (o/w) emulsion are known as m-o/w emulsions. In that emulsions, the active substance is usually hydrophobic (but can also be hydrophilic), dissolved in oil and encapsulated in a hydrophobic phase. These are central accumulation type capsules in which the droplet of the hydrophobic phase (oil) is encapsulated by being coated with a three-layer shell. Three-layer coating is achieved by triple homogenization by forming a primary, secondary and finally tertiary emulsion. In the primary emulsion, the particles of the hydrophobic phase are coated only with emulsifier particles (water-soluble ionized emulsifier with a polar end). The secondary emulsion is created by homogenizing the primary emulsion after adding a surface-active biopolymer solution (hydrocolloid 1). In the secondary emulsion, a second shell of hydrocolloid molecules 1 is formed around the emulsified particles of the fat phase. The third shell will be formed in the tertiary emulsion, i.e. by the third homogenization after the addition of the second biopolymer (hydrocolloid 2) to the secondary emulsion. With this procedure, it is possible to control the amount of encapsulated active component (it depends on the size of the droplets of the fatty phase in the primary emulsion), the thickness of the shell and the size of the capsule. In secondary and tertiary homogenization, shell porosity, shell thickness, sign and strength of capsule charge are controlled (McClements et al 2007).

The stacking of layers around the droplet of the hydrophobic phase with the active substance takes place according to the principle of electrostatic attraction. At the beginning, there are only fat droplets dissolved in the aqueous phase and emulsifier particles that coat around the particles of the fat phase. The non-polar ends of the emulsifier molecule bind to the fat droplet, and the polar ends are directed towards the aqueous phase. In this way, a barrier to the water phase is created. The fat droplet becomes encapsulated in the aqueous phase, because there is a polar outer layer of emulsifier molecules on its surface. When biopolymer particles oppositely charged to the emulsifier molecules are added to the primary emulsion, they will be attracted to the coated fat particles due to electrostatic attraction of opposite charges. The third layer is formed after the particles of biopolymer 2 are added to the secondary emulsion, the molecules of which are charged opposite to the molecules of the first biopolymer, and the process is repeated in the same way. According to the principle of electrostatic attraction, the number of layers can continue to increase.

An excess of biopolymer molecules remains in the emulsion, which failed to bind to the surface of the capsule. To finish the coating and obtain the finish surface capsule layer, it is necessary to bind the excess particles to the capsule. The finish surface layer should be the thickest. This can be achieved by adjusting the pH value, commonly by adding Ca^{2+} ions, etc. By changing the pH value, there is a change in the sign of the charge of the capsule and the capsule is again able to adsorb a new amount of biopolymer of the opposite charge. Also, there can be a change in the charge of the dissolved biopolymer, and then its particles can again bind to the oppositely charged capsule. At the end of coating, excess electrolyte is removed from the surface of the capsules (McClements et al 2007), and after that they can be spray dried.

The main advantages of encapsulation through a multilayer emulsion are reflected in the improved physical and chemical stability of the active substance and the possibility of targeted release through the gradual destruction of the structure of the multilayer shell. Natural biopolymers such as proteins, polysaccharides and phospholipids are used as coating layers. The basic prerequisite for encapsulation is that their molecules possess polar parts, ie charged functional groups. Some examples of such encapsulation carriers are casein micelles, lecithin and other phospholipids, polysaccharides (OH-groups - anions), chitosan (cations) and the like. It is considered an ideal method for the encapsulation of hydrophobic liposoluble active substances (eg different oils or some other liposoluble components) (Tahmaz 2015).

With the described procedure, it is possible to encapsulate hydrophilic substances, with the fact that the hydrophilic substance cannot be encapsulated as an integral part of the fat droplet, but as an integral part of one of the coating layers (McClements et al. 2007).

The disadvantage of using this method is that it requires great precision in order to obtain the desired thickness of the coating, but also to avoid the aggregation of the capsules.

Limonen could be encapsulated by forming a three-layer in triple emulsion. Limonene is encapsulated in a three-layer emulsion to prevent it from straightening during spray drying. The primary emulsion is formed by homogenizing the aqueous phase and oil with dissolved limonene. A hydrophilic emulsifier (proteins from lupine seeds - Lupinus album) is dissolved in the aqueous phase, the molecules of which are negatively charged. Because of that, a thin negatively charged layer of lupine protein isolate is created around the droplet of oil with dissolved limonene. The next layer from a secondary emulsion is formed when positively charged biopolymers (chitosan) are added to the primary emulsion. Chitosan binds to the free negatively charged ends of the lupine protein, causing the coated oil droplet in the secondary emulsion to become positively charged. The third layer is formed when negatively charged polysaccharides (xanthan gum, pectin and Na-alginate) are added to the secondary emulsion. Thus, in the end, the coated droplet becomes negatively charged (Burgos-Diaz et al 2018).

Ribeiro et al. (2021) used self-aggregated and cross-linked chitosan nanoparticles and maltodextrin for encapsulation of roasted coffee oil rich in phenolic compounds. Emulsion was prepared by mixing of roasted coffee oil with water suspension of cross linked and self-aggregated chitosan nanoparticles. To obtain stable capsules maltodextrin was added in prepared emulsion of coffee oil in chitosan water phase. The mixture was homogenized by high speed of ultrasonic mixer (12000 rpm for 5 minutes). Obtained emulsions were dried by freeze drying and spray drying. Particle size of dried microcapsules ranged between 10.73 and 276.21 μm. Freeze dried emulsions had significantly larger particles and higher oil retention in comparison to spray dried. Oil retention ranged 76.29 – 96.46% in freeze dried and 19.10 – 25.47 in spray dried capsules. Total phenolic content and antioxidative activity also were higher in freeze dried then in spray dried emulsions.

Application in food technology

There are many examples of application of active compounds encapsulated by emulsion based methods in enrichment of different food products. Emulsion based encapsulation techniques are very applicable for food industry. Obtained capsules can be used in original liquid emulsion form or could be additionally preserved by spray drying or freeze drying. Encapsulation of active substances in an emulsion is a very suitable encapsulation method for the food industry, and especially for products that by their very nature are emulsions. Such emulsion based products are e.g. mayonnaise, sauces, sausages, meat pates, cheese and vegetable spreads, dairy products, yogurt, cream, milk, chocolate, etc. Many of these products can be enriched with active substances encapsulated in the original liquid emulsion without drying. For other non-emulsion food products it is recommended to be enriched with previously dried emulsion based encapsulated substances.

Table 2. Examples of food products prepared with encapsulated active compounds obtained by emulsion based methods

Food type	Food product	Active compound	Coating material & emulsion method	Achieved effects
Confectionery products	Jelly and gummies	Chlorophyll from alpha-alpha	Agar and gelatin	Coloring jelly and gummy candies with green pigment chlorophyll, greater stability of encapsulated chlorophyll when heated during production (Raei et al., 2017)
	Chewing gums	Aspartame	w/o/w emulsion	Gradual/prolonged release of sweet taste
	Chocolate	Antioxidant rich moringa leaf extract	Soybean and coconut oil & w/o single emulsion	Enrichment of chocolate with antioxidants, improved stability of antioxidants from moringa leaves (Kaltsa et al., 2021)
Dairy products	Functional ice cream	*L. casei and L. lactis*	Alginate and modified starch	Improved stability of probiotic bacteria and acceptable sensory properties (Hamayouni et al., 2008)
	Cheddar cheese	Bixin	Multi-layered coating: kappa carrageenan, casein and wax & Multiple o/w/o/w	Improved stability of bixin color (Ravanfar et al., 2018)
	Melted cheese in slices	Fish oil	Milk proteins	Masked undesirable odor and taste of fish oil, acceptable sensory properties, improved oxidative stability of fish oil (Tolve et al., 2016)
	White brine cheese	*L. acidophilus. B. bifidum*	Carrageenan, Na alginate	Improved stability of probiotics (Tolve et al., 2016)
	Milk	*L. breve*	Gelatin and starch	Improved stability of probiotics in digestive tract (Tolve et al., 2016)
	Milk	Fe	Fatty acids, phospholipids	Masked metal taste, enrichment with Fe (Tolve et al., 2016)
	Yogurt	*L. acidophilus B. lactis*	Alginate and modified starch	Improved probiotic stability after 7 weeks of cold storage (4 C), mostly unchanged sensory properties, only smoothness was significantly lower in comparison to control (Tolve et al., 2016)
	Yogurt like functional beverage	Vitamin E	Yogurt based emulsion	Improved stability of vitamin E during pasteurization at 65°C, good sensory properties, increased antioxidant activity, good survival of lactic acid bacteria (Raikos et al., 2021)
Tofu	Tofu	$MgCl_2$	Alginate	Improved hardness with masked bitter taste (Tolve et al., 2016)
Ready to eat food	Mayonnaise	*L. acidophylus*	Ca-alginate and resistant starch	Better survival of probiotics, improved sensory properties (Mohammadi et al., 2013)
	Mayonnaise	*L. acidophilus, L. casei*	Ca-alginate, resistant starch	Better probiotic stability, lower

				acidity and better sensory properties (Bigdelian and Razavi, 2014)
	Mayonnaise	Walnut oil	Pectin and maltodextrin	Improved shelf life, lower peroxide number and lighter color (Akhtar et al., 2022)
	Mayonnaise	*L. casei, B. bifidum*	Resistant starch and Ca-alginate	Enhanced survival of probiotics (Fahimdanesh et al., 2012)
	Yogurt sauce	*L. paracasei*	Resistant starch and Na-alginate	Increased survival of probiotics without negative effects on color, consistency, taste and acidity (Porjavid et al., 2022)
	Soy sauce	*Z. rouxii, T. halophylus*	Alginate & w/o/w	Enhanced soy sauce aroma development during moromi fermentation (Dewanthi et al., 2018)
	Instant soup mixture	Fish oil	Skimmed milk powder & Emulsification and spray drying	Improved oxidative stability of omega 3 fatty acids, acceptable sensory properties, masking fish oiltaste (Fakir et al., 2018)
	Beef burger	*Thymus vulgaris* essential oil	Chitosan & o/w emulsion and ionic gelation	Improved antimicrobial stability and sensory properties, inhibition of discoloration (Ghandari et al., 2016)
	Beef burger	Safflower oil enriched with acai extract	Amorphophallus konjac and Na-alginate & Hydrogel emulsion technique	Extended shelf life of beef burger (Hanula et al., 2022)
	Meatballs	Cod liver oil	Na alginate and lupin protein isolate & o/w emulsion	High encapsulation efficiency 95,62%, improved heat stability of meatballs during heat treatment at 70-100C. scceptable sensory properties (Elsebaie et al., 2022)
	Fish burger	Lemon essential oil	Chitosan and modified starch	Reduced oxidation during cold storage and increased shelf life (Hasani et al., 2020)
Oils and fats	Margarine like reduced fat spreads	Beta carotene	Palm originated nanofibrilated cellulose & Pickering o/w emulsion	Enrichment of low fat margarine with beta carotene (Bernice et al., 2024)
	Margarine	Anthocyanins from rossele and red cabbage	Maltodextrin & Microwave melting of maltodextrin and encapsulation in o/w emulsion	Enrichment of margarine with anthocyanins, higher antioxidative activity of margarine, improved stability of anthocyanins against oxidation (Zaidel et al., 2014)

Bernice et al. (2024) used o/w Pickering emulsion method to produce nanofibrilated cellulose nanocapsules with beta carotene, which were used as fat replacements in formulation of low fat margarine like spreads. Obtained capsules had particle size between 64.67 and 94.73 nm, zeta potential from -32 and -38 mV and very high encapsulation efficiency 86..87 – 89.90 %. Pretty high negative values of zeta potential indicate to good particle stability against aggregation. All values (particle size, zeta potential and encapsulation efficiency) increased with increasing concentration of coating material (nanocellulosose). Obtained maragrine like spread had increased viscosity (1.83 (control without cellulose) – 10545 mPas) with increased concentration of coating material.

Low fat spreads and other low fat products are insufficient in liposoluble vitamins and provitamins content, and because of that it is desirable to be enriched with encapsulated liposoluble vitamins. Encapsulation of beta carotene improved its solubility in water and hydrophilic solutions (Tahmaz 2014).

Some examples of possible applications of emulsion based encapsulated active compounds in food technology are given in Table 2.

Kultsa et al. (2021) encapsulated antioxidants rich moringa leaf extract into single w/o emulsion containing soybean and coconut oil, and emulsifiers/surfactants Span 80 and Tween 80. In first step, oils were mixed with surfactants using pretty low speed (600 rpm) until obtain stable homogeny mixture. Then, extract started to be slowly added drop-by-drop under higher speed stirring (1000 rpm). Obtained emulsion with encapsulated extract was added to chocolate to enrich its antioxidative properties. Emulsion particle size ranged between 15.1 and 82.60 nm, and increased by increasing content of oil in emulsion.

Fish burgers produced with the addition of nanocapsules of lemon essential oil encapsulated in chitosan/starch mixture (o/w emulsion) had extended shelf life and better quality during 18 days of cold storage. Nanocapsules with essential oil improved antioxidative properties and inhibited lipid oxidation of fish burger during storage. Fish burger with encapsulated lemon oil had better overall acceptability in comparison to control.

Encapsulated probiotic bacteria L. acidophilus added to mayonnaise sauce had survived longer in comparison to free bacteria. The L. acidophilus cells are very sensitive under different factors during processing, and because of that encapsulation could serve as a promising method to protect probiotics and to improve the probiotic activity of the yogurt sauce. Besides the improved probiotic activity, yogurt sauce with encapsulated probiotics had better sensory properties (Mohammadi et al., 2013). Encapsulated bacteria L. acidophilus and L. casei reduce the overall acidity of the mayonnaise-based sauce without significant changes in consistency and rheological properties. After 90 days of refrigerator storage, encapsulated probiotic cells in sauce can remain preserved in an amount corresponding to the probiotic therapeutic minimum (Bigdelian and Razavi 2014).

Raikos et al.(2021) used emulsion method to formulated functional yogurt based drink with encapsulated vitamin E. Vitamin E was dissolved in corn oil, which represent oil phase. Yogurt base beverage was formulated by mixing of water, goat milk powder and freeze dried lactic bacteria yogurt culture. Obtained yogurt based mixture represented water phase in emulsion. Both mixtures were mixed together, by adding of oil phase slowly into water phase and stirred using high speed mixer. Obtained emulsion was fermented at 43°C. Encapsulated vitamin E had better stability in comparison control. Yogurts with encapsulated vitamin E had better survival of lactic bacteria from culture during pasteurization and storage.

CONCLUSION

Emulsions are used in almost every encapsulation technique, tha are based on dispersing of active substance in the solution of coating material. Encapsulation methods based on emulsions can be used alone or be combined with other encapsulation techniques like spray drying, freeze drying, microwave heating, high hydrostatic pressure.The most relevant characteristics of capsules obtained by emulsion based methods are:

- Capsule type:
  - central accumulation type (multi-layer coating in emulsion); and
  - matrix (encapsulation in single or double emulsion)
- Capsule size: micro or nanocapsules depending on the particle size
  - Encapsulation carriers: biopolymers, emulsifiers and other surfactants, e.g. proteins (gelatin, casein, whey proteins), chitosan, lecithin, etc.
- Advantages: good protection of the encapsulated substance from high temperatures and oxidation during heat treatment and drying, the possibility of encapsulating liposoluble and hydrosoluble substances, controlled release of the active substance.
- Disadvantages: poor encapsulation efficiency for hydrophilic active substances in the case of single-layer emulsion.
- Application possibilities: A widespread encapsulation method in industrial application, a wide range of active substances can be encapsulated, such as probiotic bacteria, proteins, amino acids, essential oils, fish oil, flavonoids, vitamin E, lutein, beta carotene, fish oil, omega 3 fatty acids, aspartame and other sweeteners, xylitol and menthol in chewing gum (prolonged cooling effect), $MgCl_2$ used in the production of tofu to mask its bitter taste, curcumin, catechin, vitamin C, vitamin B_{12} (for enrichment dairy products), vitamin B1, stevia, saffron, grape seed extracts, dyes, vitamins and minerals. The highest potential has in emulsion based or emulsion containing food products such as dairy products, sauces and dressings, mayonnaise, minced meat ready to eat products, margarine and low fat spreads.

REFERENCES

Akhtar G., Masoodi F.A., 2022. Structuring functional mayonnaise incorporated with Himalayan walnut oil. Pickering emulsions by ultrasound assisted emulsification. Ultrason. Sonochem. 86, 106022.

Ax K (2003): Emulsionen und Liposomen als Trägersysteme für Carotinoide. Doctoral dissertation, University of Karlsruhe. Shaker Verlag, Germany.

Bakry, A. M., Abbas, S., Ali, B., Majeed, H., Abouelwafa, M. Y., Mousa, A., & Liang, L. (2015).:Microencapsulation of Oils: A Comprehensive Review of Benefits, Techniques, and Applications. Comprehensive Reviews in Food Science and Food Safety, 15(1), 143–182. doi:10.1111/1541-4337.12179.

Bernice Q.Q.L., Wai Ting Chong W.T., Thilakarathna R. C. N., Tong S.C., Tang TK., Phuah ET., Yee-Ying Lee YY., 2024. Palm-based nanofibrillated cellulose (NFC) in carotenoid encapsulation and its incorporation into margarine-like reduced fat spread as fat replacer, J. Food Sci. 2024;89:5031–5046.

Bigdelian E., Razavi S.H., 2014. Evaluation of survival rate and physicochemical properties of encapsulated bacteria in alginate and resistant starch in mayonnaise. J. Bioprocess. Biotech. 4 (5), 1000166.

Burgos-Díaz, C., Hernández, X., Wandersleben, T., Barahona, T., Medina, C., Quiroz, A., & Rubilar, M. (2018): Influence of multilayer O/W emulsions stabilized by proteins from a novel lupin variety Alu Prot -CGNA and ionic polysaccharides on d -limonene retention during spray-drying. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 536, 234–241. doi:10.1016/j.colsurfa.2017.04.032.

Cheon J.,Haji F., Baek J., Wang Q., Tam K.C., 2023. Pickering emulsions for functional food systems, Journal of Agriculture and Food Research 11, 100510.

Dang, Y.T., Tran, H.; Kha, T.C., 2024. Encapsulation of W/O/W Acerola Emulsion by Spray Drying: Optimization, Release Kinetics, and Storage Stability. Foods 2024, 13, 1463. https://doi.org/10.3390/foods13101463.

Dewanthi P.V.P., 2018. Microbial encapsulation for enhancing soy sauce aroma development during moromi fermentation. Ph.D. thesis, School of Chemical Engineering, University of Birmingham.

Dubey R., Shami T.C., Bhasker Rao K.U. (2009): Microencapsulation Technology and Applications. Defence Science Journal, Vol. 59, No. 1, January 2009, pp. 82-95Ó 2009, DESIDOC.

Elsebaie E.M., Kassem M.M., Mousa M.M., Basouny M.A.M., Zeima N.M., Essa R.J., 2022. Cod liver oils encapsulation into sodium alginate/lupun protein beads and its application in functional metballs preparation, Foods 2022 11(9), 1328.

Fahimdanesh M., Mohammadi N., Ahari H., Zanjani M.A.K., Hargalani F.Z., Behrouznashab K., 2012. Effect of microencapsulation plus resistant starch on survival of Lactobacillus casei and Bifidobacterium bifidum in mayonnaise sauce. Afr. J. Microbiol. Res. 6(40), 6856–6858.

Fakir A.D., Waghmare J., 2018. Application of microencapsulated fish oil in instant soup mix as a source of omega 3 fatty acids. Int. J. PharmTech Res. 11(4), 305–313.

Fitri I.A., Mitbumrung W., Akanitkul P., Rungraung N., Kemsawasd V., Jain S., Winuprasith T., 2022. Encapsulation of ß-Carotene in Oil-in-Water Emulsions Containing Nanocellulose: Impact on Emulsion Properties, In Vitro Digestion, and Bioaccessibility. Polymers 14, 1414. https://doi.org/10.3390/polym14071414

Ghaderi G.M., Berzegar M., Sahari M.A., Azizi M.H., 2016. Nanoencapsulation approach to improve antimicrobial and antioxidant activity of thyme essential oil in beef burgers during refrigerated storage. Food Bioprocess Technol. 9(7), 1187–1201.

Hanula M., Szpicer A., Gorska-Horczyczak E., Khachatryan G., Pogorzelcki G., Pogorzelcka-Nowicka E., Poltorak A., 2022. Hydrogel emulsion with encapsulated safflower oil enriched with acai extract as a novel fat substitute in beef burgers subjected to storage in cold conditions. Molecules 27, 3–24 (MDPI).

Hasani S., Ojagh S.M., Ghorbani M., Hasani M., 2020. Nano-encapsulation of lemon essential oil approach to reducing oxidation process in fish burger during refrigerated storage. J. Food Biosci. Technol. 10(1), 35–46.

Kaltsa O., Alibade A., Batra G., Bozinou E., Makris D.P., Lalas S. I., 2021. Fortification of chocolate using Moringa oleifera extract encapsulated in microemulsions, OCL 28: 38.

Li W., Zhang L.,Ge X., Xu B., Zhang W., Qu L., Choi C.H., Xu J., Zhang A., Lee H., Weitz D.A. (2018): Microfluidic fabrication of microparticles for biomedical applications. Chem. Soc. Rev., 2018, 47, 5646—5683.

Madene A., Jacquot M., Scher J., Desobry S. (2006): Flavour encapsulation and controlled release – a review. International Journal of Food Science and Technology 41, 1–21.

Manjanna K.M., Shivakumar B. Pramod Kumar T.M., 2010. Microencapsulation: An Acclaimed Novel Drug- Delivery System for NSAIDs in Arthritis, Critical Reviews™ in Therapeutic Drug Carrier Systems, 27(6), 501–532.

Mao, L., & Miao, S. (2015). Structuring Food Emulsions to Improve Nutrient Delivery During Digestion. Food Engineering Reviews, 7(4), 439–451.doi:10.1007/s12393-015-9108-0.

McClements, D. J., Decker, E. A., & Weiss, J. (2007): Emulsion-Based Delivery Systems for Lipophilic Bioactive Components. Journal of Food Science, 72(8), R109–R124.doi:10.1111/j.1750-3841.2007.00507.x.

Mohammadi N., Ahari H., Fahimdanesh M., Zanjani M.A.K., Anvar A.A., Shokri E., 2013. Survival of alginate prebiotic microencapsulated Lactobacillus acidophilus in mayonnaise sauce. Iran. J. Vet. Med. 6(4), 259–264.

Muschiolik, G., Dickinson, E. (2017): Double Emulsions Relevant to Food Systems: Preparation, Stability, and Applications. Comprehensive Reviews in Food Science and Food Safety, 16(3), 532–555. doi:10.1111/1541-4337.12261.

Pegg R.B., Shahidi F. (1999): Encapsulation and controlled release in food preservation. In: Handbook of Food Preservation (Rahman S. ed.), Marcel Dekker Inc., pp. 611-667.

Peng, Q.; Meng, Z.; Luo, Z.; Duan, H.; Ramaswamy, H.S.;Wang, C., 2023. Effect of Emulsion Particle Size on the Encapsulation Behavior and Oxidative Stability of Spray Microencapsulated Sweet Orange Oil (Citrus aurantium var. dulcis). Foods 2023, 12, 116. https://doi.org/ 10.3390/foods12010116

Porjavid H., Ateim M., Pourhmad R., Anvar A.A., Behmadi H., 2022. Improvement of quality parameters of novel symbiotic yogurt sauce using microencapsulated Lactobacillus paracasei and natural prebiotics. Food Sci. Technol. 4, e40322, 1–12.

Prichapan N., Klinkesorn U. (2014): Factor affecting the properties of water-in oil-in-water emulsions for encapsulation of minerals and vitamins / Songklanakarin J. Sci. Technol. 652 36 (6), 651-661.

Raei A., Yasini Ardakani S.A., Daneshi M. (2017): Microencapsulation of the green pigment of alfalfa and its applications on heated food. Journal of Food Process Engineering, 40(5), e12529. doi:10.1111/jfpe.12529.

Raikos, V.; Pirie, L.P.; Gürel, S.; Hayes, H.E., 2021, Encapsulation of Vitamin E in Yogurt-Based Beverage Emulsions: Influence of Bulk Pasteurization and Chilled Storage on Physicochemical Stability and Starter Culture Viability. Molecules 2021, 26, 1504. https://doi.org/10.3390/ molecules26061504

Ribeiro H.S., Schuchmann H., Engel R., Walz E., Briviba K. (2010): Encapsulation of carotenoids. In: Encapsulation technologies for active food ingredients and food processing (Zuidam N.J., Nedović V. eds), pp. 211-253.

Ribeiro E. F., Polachini T. C., Alvim I. D., Quiles A., Hernando I., Nicoletti V. R., 2021. Microencapsulation of roasted coffee oil Pickering emulsions using spray- and freeze-drying: physical, structural and in vitro bioaccessibility studies, International Journal of Food Science and Technology 2021,

Rodríguez J., Martín M. J., Ruiz M. A., Clares B. (2016): Current encapsulation strategies for bioactive oils: From alimentary to pharmaceutical perspectives. Food Research International, 83, 41–59.doi:10.1016/j.foodres.2016.01.032.

Silva P.T., Fries L.L.M., Menezes C.R., de Holkem A.T., Schwan C.L., Wigmann É.F., Silva C. de B. (2014): Microencapsulation: concepts, mechanisms, methods and some appcations in food technology. Ciência Rural, 44(7), 1304–1311.doi:10.1590/0103-8478cr20130971.

Tahmaz J., 2014. Application of high hydrostatic pressure in modification of casen micelles and encapsulation of beta barotene, PhD Thesis. University of Sarajevo, Faculty of Agriculture and Food Sciences Sarajevo 2014.

Tahmaz J., 2023. Encapsulation and its possible application in technology of ready to eat and ready-made food, M. Brka et al. (Eds.): Agriconference 2022, LNBE, pp. 335–350. Springer Nature Switzerland 2023.

Tessaro L., Marteli-Tosi M., Sobral P.J.D.A., 2022. Development of W/O emulsion for encapsulation of “Pitanga” (Eugenia uniflora L.) leaf hydroethanolic extract: droplet size, physical stability and rheology, Food Sci. Technol, Campinas, v42, e65320, 2022

Zaidel D,N.A., Sahata N.S., Jusoh Y.M.M., Muhamad I.I., 2014. Encapsulation of Anthocyanin from Roselle and Red Cabbage for Stabilization of Water-in-Oil Emulsion “ST26943”, 2nd International Conference on Agricultural and Food Engineering, CAFEi2014”, Agriculture and Agricultural Science Procedia 2, 82 – 89.

Zuidam J.N., Shimoni E. (2010): Overview of Microencapsualtes for Use in Food Products or Processes and Methods to Make Them. In: Encapsulation Techniques for Active Foods (Zuidam J.N., Nedović V.A. eds).

PHYSICAL PROPERTIES AND FLOWABILITY PROPERTIES OF COMMERCIAL TOMATO CREAM AND TARHANA SOUP POWDERS

Jasmine Tahmaz

Faculty of Agriculture and Food Sciences, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

ABSTRACT

This study aimed to investigate the physical properties of commercial tomatosoup powders and tomato soups with tarhana noodles. Three samples of tomatocream soup without noodles and three samples of tomato soup with tarhananoodles were analyzed for different physical properties (density, viscosity, granulation, bulk density, tapped density, angle of repose, adhesiveness, foam capacity, hygroscopicity, rehydration ratio). Flowability was assessed from the valuesof the angle of repose, Hausner ratio andCarr index. Thermophysical propertieswere estimated from moisture content. Resultsshowed that tomato soup powders with tarhana noodles had thinner consistency, lowerviscosity, higher bulk and tapped densities, lower adhesiveness and lower repose angle than cream soups. Considering values of the Hausner ratio andangle of repose, it can be concluded that tomato soups with tarhana noodles hadbetter flowability and lower cohesiveness in comparison to tomato cream soupswithout noodles. Values of the angle of repose indicated that tarhana soup powdershad moderate/fair flowability, while tomato cream soup powder had very poorflowability. Samples with higher noodle content had lower angles of repose, higher flowability, higher density, and lower dispersibility. Kinematic viscosity ranged between 15.73 and 176.80 mm^2/s. Thermophysicalproperties increased by increasing of water content.

Keywords: *tomato cream soup, tarhana soup, powders, physical properties, flowability*

INTRODUCTION

Soups are widely used liquid foods madeby cooking basic and additional ingredients usually served as warm appetizers at the beginning of lunch. Industrial soups are the most commonly preserved by dehydration (drying and powdering). Soups are classified as clear and cream soups. Dehydrated soups are instant products, which could bereconstituted in warm water or boiled for 5 to 10 minutes. Commercial soups reached their popularity after the invention of the canning process in the 19th century. The main advantage of dried soup production is its long shelf life and the possibility to be stored at room temperature (Fernandez-Lopez et al. 2020). Tomato soup is prepared from mashed tomato or tomato powder with the addition of different additional ingredients, such as spices (onion, pepper, paprika, garlic, parsley), mozzarella or other cheeses, milk powder, whey, different starch, noodles. İt can be produced as cream or clear (thin soup) with or without noodles. Also, one of the very popular modern trends is the industrial production of traditional dishes, such as traditional soups and tarhana. In traditional Turkish cuisine, there are many variations of tarhana soups, made from different ingredients, depending on the region. Tarhana soup is a thick or clear soup that contains tarhana noodles, as the main ingredient. Traditionally these noodles have irregular spherical round shapes and are produced from fermentedtarhana dough. Turkish tarhana noodles are commonly prepared by mixing yogurt, cereals, and/or legume flour and different cooked vegetables such as paprika, tomato, onion and seasoned with spices like mint and paprika (Trakci et al.2004, Tahmaz et al. 2023). In Bosnia and Herzegovinian cuisine tahana soup camefrom the Ottoman Empire, and become one of the most popular dishes today. Tarhana noodles, traditional to Bosnia and Herzegovina, are prepared from ashard dense fermented dough, made from white wheat flour, eggs, and mashed tomatoes or tomato juice with or without yogurt. Prepared dough ferments at room temperature for 3–4 days, resulting in sour tarhana dough. After fermentation dough is kneaded and passed through kitchen colander holes and driedat room temperature. Bosnian tarhana soup is prepared by cooking of tarhana noodles in meat broth with an addition of salt, butter, chopped/diced tomato, and spices (black paper, dried paprika powder celery, and parsley) (Lakišić1999). Because of fermentation, traditional tarhana noodles have a pleasantsour taste and aroma specific to fermented dough. In industrial conditions, tarhana soup is commonly made from ordinary non-fermented pasta formed in a spherical-like shape similar size to traditional pasta, but without thefermentation. For that reason, the taste and aroma of industrial tarhana soups are not like traditional (Tahmaz et al. 2023). Fresh tomato fruits have the following proximate composition: moisture 94%, total solids 6%, total soluble solids 5.2%, total carbohydrates 3.41%, reducedsugars 2.96%, proteins 0.30%, fats 0.70% and ash 0.55%, fresh tomatoes also contain some nutritionally valuable bioactive compounds such as vitamin C 26.6mg%, total carotenoids 37.7 mg%, lycopene 31.82 mg% and total flavonoids 18mg/g. Lycopene is a very strong antioxidant and red pigment in tomatoes (Ramadan et al. 2021; Ali et al. 2020). Tomatoes also contain pectin, which is the mostimportant ingredient for the physical properties of tomato products. Pectins are goodfood stabilizers and emulsifiers and are widely used in the food industry as athickener (Sharma et al. 1998). Over 80% of the world-produced tomatoes are consumed in the form of processed products (Gould, 1992).

MATERIALS and METHODS

Material

Research was done on six samples of commercial dehydrated/powdered tomato soups (Figure 1), from three different producers:

- TC-1 (Vispak, Visoko Bosnia and Herzegovina) - tomato cream soup, ingredients: Dehydrated tomatoes 30%, wheat flour, maize starch, salt, hydrogenated vegetable fat, sugar, skimmed milk powder, dehydrated vegetables, spices., fat in powder: 6.6%, reconstitution data: 60g of powder in 1000 ml of water

- TC-2 (Podravka, Koprivnica Croatia) - tomato cream soup with mozzarella, ingredients: Dried tomatoes 34%, potato starch, sugar, wheat flour, salt, palm oil, corn flour, skim milk powder, mozzarella cheese powder 2.1 %, whey powder, dried celery and onion,, fat in powder 8.62%, reconstitution: 80g of powder in 750 ml of water

- TC-3 (Maggi Nestle Adriatic, Surčin, Serbia) - tomato cream soup with basil, ingredients: corn starch, dried vegetables 26 % (tomato powder 25%, beetroot powder, wheat flour, sugar, salt 10.3%, spices 3.3 %, palm oil, sunflower oil,, fat in powder: 4.5%, reconstitution: 56 g of powder in 750 ml of water.

- TT-1 (Vispak, Visoko, Bosnia and Herzegovina) - tarhana dehydrated soup, ingredients: noodles 55% (wheat flour and tomato paste), concentrate 45%

(dehydrated tomato min. 25%, potato starch, salt, hydrogenated vegetable fat, parsley, spices, flavor),fat in powder 6.66%, reconstitution instruction: 60 g of powder in 1000 ml of water

- TT-2 (Podravka, Koprivnica Croatia) - tarhana dehydrated soup with noodles and tomato ingredients: noodles 43%, salt, dried tomatoes 7.7%, wheat flour, palm oil, dried beef meat extract, red pepper, fat in powder 7.14%, reconstitution instruction: 70g of powder in 1000 ml of water

- TT-3 (Maggi Nestle Adriatic, Surčin, Serbia) - tarhana soup, ingredients: noodles 40%, tomato powder 22%, dried onion 1.7%, parsley 0.3%, other dried vegetable 2.1% (beetroot, carrot), garlic powder 0.2%, salt 11.8%, corn starch, sugar, palm oil, spices (parsley leaves, red pepper powder), fat in powder: 2.7%, reconstitution instruction: 60g of powder in 1000 ml of water.

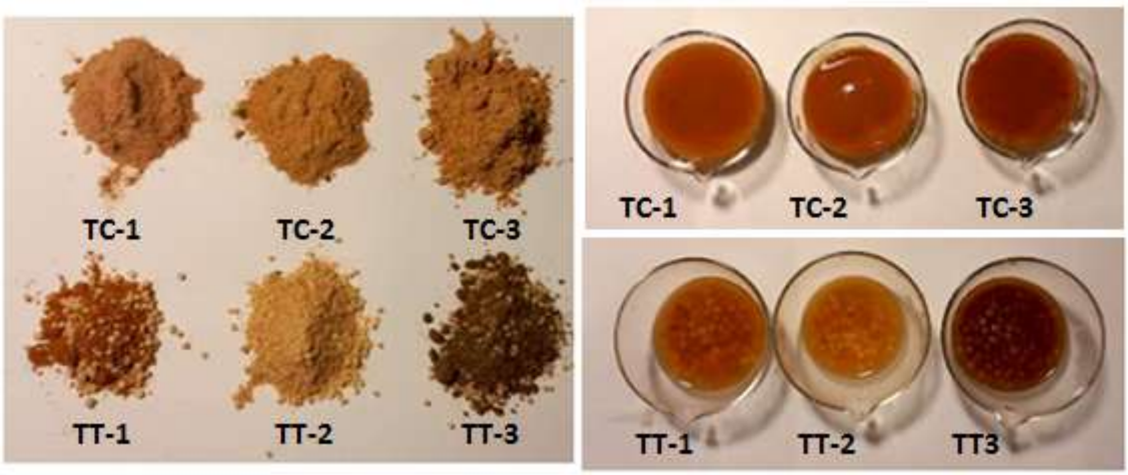


Figure 1 Image of soup powders and prepared liquid soups

Methods

Chemial analysis

Moisture content was determined by drying at 105 °C to constant weight (AOAC 1995). pH value was measured by pH meter (Mettler Toledo).

Physical analaysis of soup powders

The following characteristics were analysed on soup powders: amount and size of noodles, granulation, bulk density, tapped density, hygroscopicity, wettability, dispersibility, foam capacity and rehydration ratio.

The **amount and size of tarhana noodles** were analyzed in tarhana soup samples by sieving (Prufsieb ISO 3310–1). The size of the noodle pieces was measured by a digital caliper. 20 measurements were done on each sample, and the mean value was calculated.

For **granulation** determination, the whole amount of packed sample was sieved through sieves (PrufsiebISO 3310-1) and 5 fractions were obtained (≥ 2.5 mm, ≥ 1 mm, ≥ 0,5 mm, ≥ 0,25mm and < 0,25 mm). Determination of wettability[s] was described by Fernandes et al. (2013) with some modifications. 0.5 g of soup powder was put over the surface of 200 ml of distilled water (20 ◦C) without agitation and time was recorded after all powder had been wetted.

For **dispersibility** determination, 5 g of powder was put in a 50 ml measuring cylinder and distilled water (20 ◦C) was added to 50 ml. The mixture was vigorously stirred and left to stay for 3 h without agitation. Dispersibility [%] was calculated as a percentage of liquid phase volume in total volume (50 ml) (Alawode et al. 2017, Asma et al. 2006, El-Gindy 2018).

For **hygroscopicity** determination, 1-2 g of powder was weighted into open glass containers. Samples and saturated NaCl solution (40 g of NaCl in 100 ml of water) were left in a closed desiccator for 7 days. Hygroscopicity is calculated according to the formula (Fernandes et al. 2013):

$$\boldsymbol{Higroscopicity}\ (\%) = \frac{\boldsymbol{m_2 - m_1 - m}}{\boldsymbol{m}}\mathbf{100}$$

Where m2 is mass of container with sample after 7 days, m1 is mass of empty container and m is initial mass of sample (1-2 g).

For determination of **rehydration ratio** 5 g of powder was mixed with 50 ml of distilled water and put to boil for 2 minutes in a covered container. After boiling, the sample was filtered through weighted medical gauze and the gauze with the sample was air dried for 4 hours at room temperature (Kour et al. 2024). Rehydration ratio was calculated according to the formula:

$$\boldsymbol{Rehydration\,ratio} = \frac{\boldsymbol{m_2 - m_1}}{\boldsymbol{m}}$$

Where m_2 is the mass of rehydrated sample on the gauze, m_1 is the mass of gauze and m is initial sample mass.

Reconstitution index was calculated as weight ratio of prepared ready to eat liquid soup and soup powder used for preparation. Liquid soups were prepared according to producer instructions.

For **foam capacity** determination 2 g of the sample and 50 ml of distilled water (30 C) are put in a 100 ml measuring cylinder. The initial volume (before shaking) of the sample with water was recorded. Then the sample was mixed with water and the mixture was vigorously shaken for 3-5 minutes and the foam volume was measured. The foam capacity was calculated according to the formula (Imtiaz et al. 2007):

$$\boldsymbol{Foam\ capacity}\ (\%) = \frac{\boldsymbol{V_{foam}}}{\boldsymbol{V_{initial}}}\mathbf{100}$$

Bulk density was determined as ratio of weight and volume of powder in measuring cylinder. Sample powders were poured gently into a 50 ml graduated and tarred measuring cylinders. Bulk density was calculated as ratio of sample weight and volume (Fernandes et al. 2013).

Tapped density was determined after bulk density determination. In the same measuring cylinder soup powders were tapped 100 times (or to constant volume) by beating on a plain surface. The new tapped volume was recorded, and tapped density was calculated as ratio of sample weight and tapped volume (Fernandes et al. 2013, Imtiaz et al. 2007).

Table 12 Referent values of Carr index, Hausner ratio and angle of repose for flowability estimation

Carr index	Hausner ratio	Angle of repose (degrees)	Flowability
≤10	≤1.11	≤30	Excellent, easy flow
11-15	1.12-1.18	31-35	Good
16 -20	1.19-1.25	36-40	Fair
21-25	1.26-1.34	41-45	Passable
26-31	1.35-1.45	46-55	Poor
26-31	1.35-1.45	46-55	Poor
32-37	1.46-1.59	56-65	Very poor, limited
>38	>1.60	>66	Extremely poor
Carr index	Hausner ratio	Angle of repose (degrees)	Flowability

Szulz and Lenart (2016, Jan et al. (2015)

Flowability and cohesiveness

Angle of repose, Carr index and Hausner ratio were used as indicators of flowability and cohesiveness of soup powders. For determination of angle of repose, 50 ml of dehydrated soup sample powder was carefully sloped through a funnel (D = 1 cm) on a plain surface base. The height (h) and diameter (d) of the powder heap were measured. Angle of repose α was calculated as:

$$\mathbf{tg\ \alpha} = \frac{\boldsymbol{2h}}{\boldsymbol{D}}$$

Dynamic angle of repose was calculated as 70% of angle of repose.

The Hausner ratio and Carr index were calculated from bulk (ρB) and tapped (ρT) densities using following formulas (Szulz and Lenart 2016):

$$\boldsymbol{Hausner\ ratio} = \frac{\boldsymbol{\rho}}{\boldsymbol{\rho_B}}$$

$$\boldsymbol{Carr\ index}\ (\%) = \frac{\boldsymbol{\rho_T} - \boldsymbol{\rho_B}}{\boldsymbol{\rho_T}}\mathbf{100}$$

Carr index and Hausner ratio were compared to referent values (Szulz and Lenart 2016, Jan et al. 2015; Sahni and Shere 2017), and flowability was described in comparison to Table 1.

Physical analysis of reconstituted soups

Liquid soup samples were cooked according to producer instructions. Density of liquid samples was calculated as ratio of measured weight and volume of liquid soup in measuring cylinder. Determination of density was done at 5, 25, 55 and 100 C.

Measurement of kinematic viscosity was performed at 55°C by capillary viscometer Micro Ostwald 516 30/III and calculated as product of viscometer constant and time (s) needed

to soup sample pass through capillary. Dynamic viscosity (apparent viscosity) was calculated as product of kinematic viscosity and density at 55C. Apparent fluidity was calculated as reciprocal value of dynamic viscosity (Hlavač et al. 2019; Sing and Heldman 2003).
Determination of adhesiveness was done by method described by Sihshobhon et al. (2013) with some modifications. 100 g of prepared soup samples was placed into laboratory glass, and wooden stick (15x1.7x0.1 cm) was immersed into samples, taking care that 10 cm of stick should be immersed. Adhesiveness was calculated by formula:

$$\boldsymbol{Adhesiveness}\ (\%) = \frac{\boldsymbol{m_2 - m_1 - m}}{\boldsymbol{m}}\boldsymbol{100}$$

Where are: m_2 is weight of wooden stick with sample, m_1- initial weight of wooden stick without sample and m – weight of sample in glass.
Thermophysical properties were estimated by different mathematical models from moisture or total solids content in liquid samples.
Freezing point was calculated by Guegov`s model applicable for food with solids between 3.50 and 27% (Guegov 1980):

$$\boldsymbol{Freezing\ point}\ (°\boldsymbol{C}) = \boldsymbol{0.36 - 0.175}\ \%\boldsymbol{solids}$$

Specific heat capacity (cp) was calculated by model established by Dickerson for liquid or semiliquid food (>50% of water) (Delgado et al. 2006):

$$\boldsymbol{c_p(J/kgK) = 1674.7 + 25.12}\ \%\boldsymbol{water}$$

Bowman`s model was used for prediction of thermal conductivity coefficient:

$$\lambda(\boldsymbol{W/mK}) = \boldsymbol{0.056 + 0.567}\ \%\boldsymbol{water}$$

Thermal diffusion coefficient was calculated from general definition equation, which assumed density, thermal conductivity and specific heat capacity (Singh and Heldman 2003):

$$\boldsymbol{\alpha}\ (\frac{\boldsymbol{m^2}}{\boldsymbol{s}}) = \frac{\boldsymbol{\lambda}}{\boldsymbol{\rho\ c}}$$

where α is thermal diffusion coefficient [m2/s], λ is thermal conductivity calculated by Browman`s model [W/(mK)], ρ is measured density at 25C [kg/m3] and Cp is calculated specific heat capacity [J/(kgK)].
Statistical analysis: was performed by one-way ANOVA and Tukey test ($p \leq 0.05$) using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). All analysis was done in triplicate and results were shown as mean ± standard deviation (SD).

RESULTS and DISCUSSION

Chemical anlysis

Results of chemical analysis are given in Table 2. In soup powders moisture content ranged between 5.56 and 8.89% without significant differences between samples. Tarhana soups had slightly lower moisture content in comparison to cream soup powders, but differences were not significant ($p \leq 0.05$). All samples had moisture content lower than 10%, which is recommended for dry food. Obtained results were in agreement to literature data for similar soup samples (Bhargavavanadha et al. 2021; Goncu and Celik 2020, Cagindi et al. 2016, Ansari et al. 2021; Tahmaz et al. 2023, Celik et a. 2010, Verma and Mogra 2017, Gandhi, et al. 2017, Kambabzi et a. 2020). Reported moisture content commonly ranged between 5.30 and 10. 95% in dry tarhana soups (Tahmaz et al. 2023, Goncu and Celik 2010, Celik et al. 2010, Dagtekin and Misir 2023, Cagindi et al., 2016), while between 5.14 and 9.04% in tomato cream soup powders (Bhargavanandha et.al. 2021, Verma and Mogra 2017, Gandhi et al. 2017). Literature data also reported that industrial tarhana soup has lower moisture content in comparison to homemade and experimentally produced (Tahmaz et al 2023, Celik et al., 2010). Moisture content in prepared liquid soup ranged between 87.85 and 94.52%. According to Gawad et al. (2022) moisture content in liquid cream soup was 87.5%, which is similar to sample TC-2 (tomato cream soup with mozzarella).

pH varied between 4.25 and 5.20 (Table 2). Tarhana soup TT-1 had significantly the highest pH value in comparison to other samples. Obtained results are in agreement to literature data (Hassan and Gadallah 2018, Ertop et al. 2019, Celik et al., 2010, Koc and Ozgire, 2019, Goncu and Celik 2020, Cagidri et al. 2016, Tahmaz et al. 2023). Reported pH values ranged between 3.20 and 5.70 for tomato cream soup (Jamshidvand et al. 2023, Sharma et al. 2023, Bhargavanandha et al. 2021, Gawad et al. 2022) and between 3.6 and 6.0 for tarhana soups (Hassan and Gadallah, 2018, Ertop et al. 2019, Celik et al. 2010, Koc and Ozgira 2019, Goncu and Celik 2020, Cagidri wt al. 2016, Tahmaz et al. 2023, Gohari 2022) depending on formulation.

Table 2 Results of chemical properties

Samples	Moisture in soup powders (%)	Moisture in liquid ready to eat soups (%)	pH
TC-1	6.76±0.33	94.52±0.42	4.65±0.07[ab]
TC-2	8.89±0.54	87.85±8.41	4.90±0.14[ab]
TC-3	6.42±0.84	93.36±0.25	4.40±0.14[ab]
TT-1	6.42±1.86	91.06±0.27	5.20±0.28[a]
TT-2	5.56±0.37	92.83±2.10	4.90±0.14[ab]
TT-3	7.77±2.09	94.06±1.12	4,25±0.35[b]

*Different small letters in columns represent statistically significant differences ($p \leq 0.05$) between samples

Table 3 Amount and size of noodles

Samples	Amount of noodles (%)	Noodle size (mm)
TT-1	55.32±0.18[a]	2.45±0.84[a]
TT-2	49.77±2.05[a]	2.01±0.51[ab]
TT-3	43.23±1.44[b]	1.74±0.44[b]

*Different small letters in columns represent statistically significant differences ($p \leq 0.05$) between samples

Physical anlysis

Amount of noodles in tarhana soups ranged between 43.23 and 55.32% (Table 2, Figure 2). TT-3 sample had significantly lower amount of noodles in comparison to other samples. Tahmaz et al. (2023) reported 55.99% of noodles in tarhana soup from the same producer like TT-1, which is very close to results for TT-1.

Average tarhana noodle size ranged between 1.74 and 2.46 mm (Table 3). TT-1 sample had the largest noodles, while TT-3 had the lowest. Noodles had cylindrical shape (unlike traditional tarhana), which was obtained by cutting of cylindrical extruded noodle dough (Figure 3). Traditional tarhana noodles commonly have rough irregular spherical/rounded shape with different sizes. All pieces of traditional tarhana have smooth rounded edges and sides without sharp parts. Industrial noodles from analyzed samples were not real tarhana noodles produced from fermented dough. As a metter of fact, these noodles were tarhana-like noodles produced from ordinary nonfermented dough. It can be seen (Figure 3) that TT-1 sample had the largest noodles with porous surface, while other samples had smooth compact surfaces without any ruptures. TT-2 and TT-3 had smaller and more uniform noodles in comparison to TT-1.

Figure 2 Image of powder and noodle fraction in tarhana soup samples

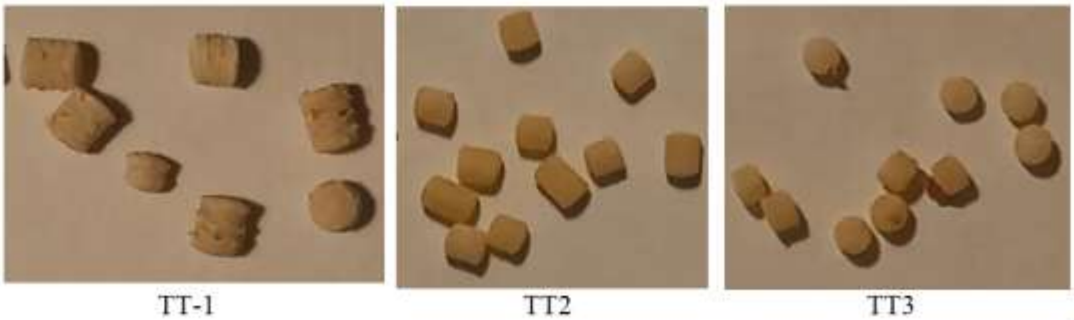


Figure 3 Shapes of the noodles

The results of the granulation analysis are given in Table 4. Cream soup samples had significantly the largest amount of very small particles (< 0.25 mm), while tarhana soups had significantly the largest amount of large particles (>1 mm). Tomato cream soups had extremely highest amount of total small particles (76-91,33%), while tarhana soup samples had more or less similar amounts of the total large (41.98-47,12%) and the total small particles (40.81-55.35%). All samples contained the lowest amount of medium particles. Particles larger than 2.5 mm were pretty low presented in all samples, and noodle sizes were mostly between 1 and 2.5 mm. Large particles (>1 mm) consisted of tarhana noodles in tarhana soup samples, while very large particle fraction (>2mm) mostly consisted of powder lumps and small amounts of dried vegetables (Figure 2). The highest amount of powder lumps was noticed in the TT-3 sample. Considering cream soups the highest amount of powder lumps was noticed in sample TC-2, which has the highest level of large particles (13.99%) in comparison to other cream soups (3.26-3.70%). Very large particle fraction (>2.5 mm) in all samples was the least represented fraction, which mostly contained powder lumps or some pieces of stuck or oversized noodles. Medium and small particle fractions mostly contained salt crystals and small pieces of dried green vegetables and spices.

Table 4 Results of granulation analysis (%)

Samples	Very large ≥ 2.5 mm	Large ≥1 mm	Medium ≥ 0.50 mm	Small ≥ 0.25 mm	Very small < 0.25 mm
TC-1	0.40±0.14^{c}	2.86±0.63^{b}	5.41±0.59^{cd}	2.71±0.9^{a}	88.63±0.29^{a}
TC-2	6.71±1.15^{a}	6.88±1.40^{b}	10.34±0.35^{ab}	6.28±3.27^{b}	69.73±0.48^{b}
TC-3	1.28±0.08^{bc}	2.42±0.39^{b}	8.23±0.66^{bc}	3.15±1-65^{b}	87.97±5.78^{a}
TT-1	9.07±1.92^{a}	38.80±4.11^{a}	11.33±1.23^{a}	17.09±3.21^{a}	23.72±4.06^{e}
TT-2	0.75±0.35^{bc}	46.37±1.16^{a}	5.81±0.30^{cd}	3.78±1.25^{b}	43.18±2.63^{d}
TT-3	5.35±1.88^{ab}	36.63±4.16^{a}	4.58±0.83^{d}	3.81±1.71^{b}	51.54±6.19^{c}
Samples	**Large ≥1 mm**		**Medium ≥ 0.50 mm**	**Small ≥ 0.25 mm**	
TC-1	3.26±0.77^{b}	5.41±0.59^{cd}	91.33±0-18^{a}	3.26±0.77^{b}	5.41±0.59^{cd}
TC-2	13.59±2.55^{b}	10.34±0.35^{ab}	76.00±2.79^{a}	13.59±2.55^{b}	10.34±0.35^{ab}
TC-3	3.70±0.47^{b}	8.23±0.66^{bc}	91.12±4.13^{a}	3.70±0.47^{b}	8.23±0.66^{bc}
TT-1	47.86±6.02^{a}	11.33±1.23^{a}	40.81±7.27^{b}	47.86±6.02^{a}	11.33±1.23^{a}
TT-2	47.12±1.51^{a}	5.81±0.30^{cd}	46.96±1.38^{b}	47.12±1.51^{a}	5.81±0.30^{cd}
TT-3	41.98±6.04^{a}	4.58±0.83^{d}	55.35±7.90^{b}	41.98±6.04^{a}	4.58±0.83^{d}

*Different small letters in columns represent statistically significant differences ($p \leq 0.05$) between samples

The smallest fraction (<0.25 mm) in all samples contained only powder (mostly tomato powder and starch). İt can be seen that samples differed significantly in each fraction. Sample TC-1 had the highest amount of very small particles, while TT-1 had the highest amount of very large particles. The sample with the highest amount of tarhana noodles had the highest amount of very large particle fractions. When comparing tarhana soup samples, it can be seen that the sample with the lowest noodle content (TT-3) had the lowest amount of total large particles (41.98%) and a higher amount of very large particle fraction in comparison to other tarhana samples. This can be explained by observing the highest content of powder lumps in TT-3 samples (Figure 2). Because of the higher presence of powder lumps (Figures 1 and 2), TT-1 and TT-3 had higher amounts of very large particles larger than 2.5 mm. Samples with the highest amount of very large particles (>2.5 mm) were TC-2, TT-1 and TT-3. These three samples also had the highest moisture content (Table 1). The presence of high amount of lumps in these samples could be explained by high moisture content. A high lever of moisture probably caused the sticking of powder particles and the formation of the lumps. The medium fraction was the most dominant in TC-2 and TT-1.

In comparison to other cream soup samples, tomato soup with mozzarella (TC-2) also had the largest amount of small particles (between 0.25 and 0.5 mm), but also the smallest amount of very small particles below 0,25 mm. This could be explained by the presence of mozzarella powder, which could be assumed to have larger particles than starch and other powders. This sample had the highest sum of medium and small particles (16.62%). According to the producer specification (Angelstarch 2024), tomato cream soup should contain a minimum of 65% of fine particles with a size below 0.25 mm. Results obtained for cream soups (Table 4) are in agreement with the literature (AngelStarch 2024).

Bulk density (Table 5) ranged between 464.6 and 775.4 kg/m^3. Tarhana soups had significantly ($p \leq 0.05$) higher values (641-755.4 kg/m^3) of bulk density in comparison to tomato cream soups (464.6-524 kg/m^3). Because of the noodle's compact structure, the presence of tarhana noodles significantly ($p \leq 0.05$) increased bulk density. The sample with the lowest moisture content (TT-2) had the highest bulk density. On the other hand, samples with the lowest bulk density (TC-1 and TC-3) had the highest amount of small powder particles. According to literature data, bulk density varied in ranges 450-790 kg/m^3 for tomato cream soups (Verma and Mogra 2017, Bharagavanandha et al 2021; Kour et al. 2024), and 621 – 948 kg/m^3 for tarhana soups (Koc and Ozcira 2019; Tahmaz et al. 2023). The obtained results were in agreement to literature data, which mostly reported that tarhana soups (and other soups with noodles) had higher bulk density in comparison to tomato and other cream soups.

Table 5 Results of bulk density, tapped density and hygroscopicity of analysed soup powders

Samples	Bulk density (kg/m^3)	Tapped density (kg/m^3)	Hygroscopicity (%)
TC-1	464-60±19.18^c	748.60±1.13^c	25.44±8.76
TC-2	524.30±17.39^c	809.78±4.60^b	25.59±3.08
TC-3	472.50±7.50^c	801.23±31.91^{bc}	21.16±6.35
TT-1	652.80±40.73^b	858.22±12.73^b	20.79±2.16
TT-2	755.40±7.92^a	981.21±7.99^a	21.25±0.66
TT-3	641.00±13.01^b	840.15±10.87^b	21.10±1-55

*Different small letters in columns represent statistically significant differences ($p \leq 0.05$) between samples

Tapped density (Table 5) varied in the range 748.60-981.21 kg/m^3. Tarhana soup powders had higher tapped density. Soups with tarhana noodles had higher tapped density. Samples with the highest and the lowest bulk densities had also the highest and lowest tapped densities. Similar results were obtained by other authors, for example, 866-975 kg/m^3 in tarhana soup powders (Koc and Ozcira 2019, Tahmaz et al. 2023).

Hygroscopicity (Table 5) ranged between 20.79 and 25.44% without significant differences between samples. Tomato cream soups had slightly higher hygroscopicity than tarhana soup. Samples with the highest hygroscopicity (TC-1 and TC-2) also had the lowest values of bulk density, low amount of large particles and high amount of small particles (Table). A higher amount of noodles did not have a significant influence on hygroscopicity decreasing, although TT-1 sample had the largest noodles content and the lowest hygroscopicity in comparison to other tarhana soup samples. According to previous research (Koc and Ozcira 2019; Elrih and Ismael 2020), the hygroscopicity of dried soups ranged between 0.6 and 10%, depending on the sample type and determination method. Lower values obtained in previous research can be explained by some differences in determination methodology and in shorter testing time. Koc and Ozcira (2019) reported that tarhana soup samples had hygroscopicity of 3.1 - 10%.

Powder flowability assessment

Hausner ratio, Carr index and angle of repose were used as indicators of the flowability and cohesiveness of soup powders (Table 6). Higher values indicate lower powder flowability. Powders with low flowability are more cohesive and more compressible. Hausner ratio and Carr index had values of 1.30 - 1.70 and 23.01 - 41.00. These values indicate pretty poor flowability properties. There were no significant differences between cream soup samples and between tarhana soup samples. The lowest flowability had TC-3 sample (tomato cream soup with mozzarella), which indicated very high cohesiveness and extremely poor flowability. This sample had a high amount of moisture content, the highest amount of small particles, a low amount of large particles, low bulk density but high tapped density, the highest angle of repose and the lowest wettability time. This sample also contained the visible amount of powder lumps in the large particle fraction. Samples TC-2 and TC-3 contained powder lumps larger than 2.5 mm. Both indicators were higher in tomato cream soup powders than in tarhana soups. The lowest value was noticed in the TT-2 sample. This sample had the lowest moisture content, the highest tapped density, the lowest angle of repose, the highest amount of large particles between 1 and 2 mm, the lowest viscosity, and the highest fluidity, low rehydration ratio, and adhesiveness. Between tarhana soup powders TT-2 had the highest bulk and tapped density and the smallest noodle size. All tarhana soup samples had very similar values of the Hausner ratio and Carr index. If observing only tarhana soups, the lowest flowability was in TT-1, although this sample contained the highest noodle content. Considering the values of the Hausner ratio and Carr index, the flowability of tarhana soup powders can be assessed as passable with normal (medium) cohesiveness. On the other hand, the flowability of tomato cream soups was recognized as much weaker and estimated as poor to extremely poor with high cohesiveness. Samples with higher noodles content did not show especially more intense flowability, considering that values of Carr index and Hausner ratio between tarhana soup powders were very similar.

Table 6 Hausner ratio, Carr index and flowability assessment of soup powders

Samples	Hausner ratio	Carr index	Flowability
TC-1	1.61 ± 0.07^{a}	37.94 ± 2.47^{a}	Extremely poor
TC-2	1.55 ± 0.04^{ab}	35.26 ± 1.78^{ab}	Very poor
TC-3	1.70 ± 0.04^{a}	41.00 ± 1.41^{a}	Extremely poor
TT-1	1.32 ± 0.10^{bc}	23.89 ± 5.85^{bc}	Slightly fair to passable
TT-2	1.30 ± 0.02^{c}	23.01 ± 1.40^{c}	Slightly fair to passable
TT-3	1.31 ± 0.04^{bc}	23.69 ± 2.54^{bc}	Slightly fair to passable

*Different small letters in columns represent statistically significant differences ($p \leq 0.05$) between samples

Values for the Hausner ratio and Carr index were in agreement with the literature data (Koc and Ozcira 2019, Kumari et al. 2023, Bhargavanandha et al. 2021, Tahmaz et al. 2023). Reported values were in ranges 1.02-1.57 for the Hausner ratio and 23.67-34 for the Carr index. According to Tahmaz et al. (2023), commercial tarhana soup powder had a lower value of Hausner ratio in comparison to cream soups (1.39 vs. 1.57). Bhargavanadha et al. (2023) reported lower values (1.28-1.33) for tomato powder and tomato cream soups probably because of different compositions, which are mostly reflected in the lower starch content in the formulation. Instead of starch, the mentioned authors used gum Arabica in different concentrations. Koc and Ozcira (2019) also obtained lower values (Hausner ratio 1.02-1.05 and Carr index 2.02-4.8) in the estimation of flowability and cohesiveness of tarhana noodles, which indicated better flowability and lower cohesiveness. Since analyzed tarhana soup samples contained powdered fraction (consisting of starch and tomato powder) together with noodles, obtained results were expected Because of that the flowability of soup mixtures was lower in comparison to tarhana noodles reported by Koc and Ozcira (2019). The most similar values to the obtained results were reported by Kumari et al. (2023) for cream soups and Tahmaz et al. (2023) for commercial tarhana soup mixture and cream soups. According to Kumari et al. (2023), cream soup powders had a Carr index of 26.36-34 and a Hausner ratio of 1.35-1.52, which indicated poor to very poor flowability. Tahmaz et al. (2023) also reported that tarhana soup mixtures had higher flowability (Hausner ratio 1.39) and lower cohesiveness in comparison to cream soups (Hausner ratio 1.56-1.57). According to those values flowability of tarhana soup powder was estimated as poor, while the flowability of cream soup powder was estimated as very poor.

The angle of repose ranged between 36.16 and 43.10 degrees (Table 7). Tomato cream soups had a higher angle of repose. The presence of tarhana noodles helped the powder to be loose and have a lower angle. The highest value was noticed in TC-3 and the lowest in TT-2 soup. Considering tarhana soup samples, it is obvious that samples with the lowest angle of repose had the highest noodles content, the highest amount of large particles, and the highest tapped density. On the other hand, tomato cream soup TC-3 had the highest angle of repose, the lowest bulk density and hygroscopicity. Between tarhana soup samples, the highest angle of repose was in TT-3 and the lowest was in TT-2. TT-3 had visible powder lumps in the large particle fraction (Figure), the highest moisture content, the lowest bulk density, tapped density and hygroscopicity, the highest amount of small particles and the lowest amount of noodles. All these characteristics caused the highest cohesiveness and the lowest flowability in comparison to other tarhana soup powders. Lower bulk density could be explained by less heavy powder particles, which easily stand on the top of the heap. Higher moisture content makes powder more sticky, which can contribute to the formation of a higher pile and increase the angle of repose. All these facts could contribute to higher cohesiveness and lower flowability. In the tarhana soup powders, the noodles are the heaviest fraction with the highest specific gravity/density. This fraction shows a higher tendency to be loose and scatter. Because of that, tarhana soup powders with higher noodle content had a lower angle of repose and better flowability. The angle of repose values indicated that the flowability of tomato cream soup powder can be assessed as fair to passable (with needed hang-up), while tarhana soup powders are fair without the need to be aided or agitated.

Table 7 Angle of repose and flowability assesment of soup powders

Samples	Angle of repose (degrees)	Dynamic angle of repose (degrees)	Flowability
TC-1	39.44±2-55^{abc}	27.01±1.79^{abc}	Fair, aid not needed
TC-2	42.09±1.47^{ab}	29.40±1.03^{ab}	Passable, may hang up
TC-3	43.10±1.65^{a}	30.17±1.16^{a}	Passable, may hang up
TT-1	37.08±0.14^{bc}	25.90±0.10^{bc}	Fair, aid not needed
TT-2	36.16±0.57^{c}	25.31±0.40^{c}	Fair, aid not needed
TT-3	39.16±1.24^{abc}	27.41±0.87^{abc}	Fair, aid not needed

*Different small letters in columns represent statistically significant differences ($p \leq 0.05$) between samples

The obtained values are in agreement with literature data for tomato soups and similar powders. Reported values for tomato cream soup ranged between 34 and 47 degrees (Bhargavananha et al. 2021), while for vegetable cream soup from 34.17 to 57 degrees (Tahmaz et al. 2023, Elrih and Ismael 2020, Kamble et al. 2019; Yadav et al. 2022). Tahmaz et al. (2023) reported that tarhana soup had a lower angle of repose in comparison to traditional cream soups, which is in agreement with the results obtained in this study (Table 7).

Physical properties related to soup powders ability to be dissolved

Dispersibility, wettability, foam capacity, rehydration ratio and reconstitution index are the most relevant physical properties related to soup powders ability to be dissolved and reconstituted. The highest values of dispersibility, rehydration ratio and the lower values of wettability time indicate better reconstitution. Results are presented in Table 8.

Wettability time (Table 8) varied in very huge range between 6.28 and 476.29 sec. The amount and the nature of powder fraction have a crucial influence on wettability time, while noodles represent the heavy fraction which fails very easily and fast. Because of that, samples with tarhana noodles (except TT-1) had a shorter wettability time in comparison to cream soups. Significantly the highest wettability time was noticed in TT-1. Such a long wettability time can be related to the combination of different factors (pretty high-fat content, the highest amount of medium particles with non-heavy pieces). A higher amount of low heavy (less heavy) compounds (fat and dried spices in medium fraction) probably could cause a long wettability time. This sample hadn't only the longest wettability time, it also had the lowest dispersibility and rehydration ratio. Besides that, the two samples with the longest wettability time (TT-1 and TC-2) had the lowest fat content and potato starch in their composition, unlike other samples. TC-3 and TT-3 samples had the lowest wettability time and a high amount of small particles. The obtained results were in agreement to the literature. Wettability time in soup powders commonly ranged between 1.16 and 585.8 s, depending on the soup type. It is not uncommon that tarhana soup powder has a long wettability time. According to Tahmaz et al. (2023), the Commercial tarhana soup sample (the same sample as TT-1) also had an extremely high wettability time (585.62

s) in comparison to other samples. Koc and Ozcira (2019) also reported medium to long wettability time for tarhana soups which varied in the range of 16.25 -385.5 s. The reported wettability for different tomato cream soup powders was 48-55 s (Bhargavanandha et al. (2021) and 47.23-96.26 s (Verma and Mogra (2017). Lower values (116 – 220 s) were reported for vegetable cream soups (Elrih and Ismael 2020; Bader et al. 2022, Ocieczek and Palich 2007).

Table 8 Results of physical properties related to powder ability to be dissolved in water (wettability, dispersibility, foam capacity, rehydration ratio and reconstruction index)

Samples	Wettability (s)	Dispersibility (%)	Foam capacity (%)	Rehydration ratio	Reconstitution index
TC-1	39.80±7.79^{bc}	65.50±3.54^{bc}	15.78±4.56^{a}	3.81±1.42	17.66
TC-2	56.63±6.33^{b}	82.50±3.54^{a}	15.71±1.27^{a}	5.52±1.53	10.37
TC-3	6.28±2.06^{c}	70.00±2.83^{abc}	14.02±0.79^{a}	4.34±1.16	14.39
TT-1	476.29±28.20^{a}	59.00±1.41^{c}	4.86±1.44^{b}	2.82±1.02	17.66
TT-2	14.52±1.30^{c}	75.00±1.41^{ab}	7.11±2.20^{ab}	2.89±0.65	15.28
TT-3	6.90±0.54^{c}	76.00±5.66^{ab}	4.83±1.34^{b}	3.07±1.19	17.66

*Different small letters in columns represent statistically significant differences ($p \leq 0.05$) between samples

All soup samples showed good dispersibility, with values of 59 - 82.50% (Table 8). Dispersibility of tarhana soups ranged between 59 and 76%, while in tomato cream soups 65.5 and 82.5%. Tomato cream soups had slightly higher dispersibility values than tarhana soups. Tomato soup with mozzarella (TC-2) had the highest dispersibility. Between cream soup samples, this sample had the highest amount of medium particles, the highest amount of large particles and the lowest amount of small particles, a high angle of repose, and pretty low bulk density in comparison to other samples. Low bulk density probably helps the sample to disperse better. Sample TT-1 with the lowest amount of small particles, the highest amount of noodles, the longest wettability time and the lowest hygroscopicity had the lowest dispersibility. The obtained results were in agreement with the literature. According to Koc and Ozgira (2019) dispersibility of tarhana soup ranged between 31.45 and 84.56%, Tahmaz et al. (2023) reported dispersibility of 59%, in commercial tarhana soup, which was very close to the result obtained for TT-1 sample. Other literature data give results of dispersibility in vegetable cream soups, which ranged between 57 and 82% (Elrih and Ismael 2020; Mohajan et al. 2017, SSepuya et al. 2018, Tahmaz et al. 2023).

Tomato cream soups had significantly higher foam capacity (Table 8) in comparison to tarhana soups. All samples had pretty low foam capacity. The highest foam capacity was noticed in TC-1 and TC-2. TC-1 sample had the highest amount of very small fine particles (<0.25 mm), which have crucial importance in foam stabilization, while TC-2 had the highest dispersibility. The lowest amount of foam capacity was obtained in the TT-1 sample, which had the longest wettability time and the lowest dispersibility. Foam capacity had values of 4.53-15.78%. These values were in agreement with literature data (2.13-15%), reported for vegetable cream soups (Singh and Kaur 2020, Kambabazi et al. 2022, Abd-Elhak and Salem 2017). Gohari 2022 reported an extremely high value of foam capacity for tarhana soup (43%), which could be explained by differences in tarhana soup preparation. The tarhana prepared in the mentioned research (Gohari 2022) was real tarhana prepared from fermented dough, and because of that had higher foam capacity and more stable foam. Tarhana soup samples analyzed in this study are commercial soup samples that are prepared from ordinary nonfermented noodles. It is well known that fermented products have ability to form foam.

Rehydration ratio showed values between 2.82 and 5.52 (Table 8), without significant differences between samples. The highest values were noticed in samples with the highest viscosity values. Tomato cream soups had a higher rehydration ratio in comparison to tarhana soups. Tarhana soups with higher amounts of noodles had lower rehydration ratios. According to the literature data rehydration ratio for different soups had values 2.5-6.5 (Ansari et al. 2021, Bhargavanandha et al. 2021, Abdel Haleem and Omran 2014; Bader et al. 2022), which is very similar to the obtained results. Bhargavananha et al. (2021) reported that tomato cream soup had rehydration ratio values of 3.18-3.65, which is very close to the results obtained in this study (except TC-2 and TC-3). Cream soup samples TC-2 and TC-3 had slightly higher rehydration ratio values than reported by Bragavanandha et al. (2021). These low differences could be explained by differences in tomato soup composition, viscosity and other properties. Bhargavanandha et al. (2021) reported lower viscosity values for tomato cream soups (10-49.1 mPas), while the viscosity of TC-2 and TC-3 samples had much higher values (131-176 mPas). Samples with lower viscosity (15.66-30.28 mPas) had a rehydration ratio more similar to those reported by Bhargavanadha et al. (2021). Samples with higher viscosity commonly had higher total solids content, and because of that probably had a higher rehydration ratio. Samples with a higher reconstitution index had a lower rehydration ratio.

The reconsttution index of commercial soup samples was calculated from instructions for preparation. Values ranged between 10.37 and 17.66. Higher values mean higher water content. Because of that samples with higher moisture content in the liquid soup had a higher reconstitution ratio. Tomato cream soup with mozzarella (TC-2) had the lowest reconstitution index. This sample had the highest values for total solids content, dynamic viscosity, adhesiveness and density. Higher total solids increase the values of reconstitution ratio, viscosity, and density. On the other hand, higher viscosity values increase the adhesiveness and rehydration ratio. According to Ansari et al. (2021), the reconstitution ratio in the cream soup had values between 14.26 and 16.33. Jamshidvand et al. (2023) reported a reconstitution ratio of 10.88 in tomato cream soups. Analyzed samples had values of reconstitution ratio very similar to those reported by Ansari et al. (2021) and Jamshidvand et al. (2023).

Physical properties of liquid soup samples

Results of the physical properties of reconstituted ready-to-eat soups are given in Table 9.

Kinematic viscosity had values of 15.73-176.80 mm2/s, while dynamic viscosity was 15.66 – 176.18 mPas, Tarhana soup samples had significantly lower viscosity in comparison to cream soups. The highest viscosity was in tomato cream soup with mozzarella (TC-2). This sample had the highest content of dry matter, the highest dispersibility, adhesiveness, rehydration ratio, and the highest amount of powder used for preparation. Similar values were reported by other authors. Apparent viscosity commonly ranged between 5 and 298 mPas for similar soup samples depending on temperature and sample type. Reported values for tomato cream soups were 15-298 mPas (Chawan et al., 2015, Verma and Mogra, 2017, US FDA, 2008; Skotnicka and Palich 2019, Jamshivand et al. 2023), and for tarhana soups 5-280 mPas (Tahmaz et al. 2023, Ertop et al. 2019, Hassan and Gadallah 2018). Values for apparent viscosity (Table) varied in ranges 25.0-176.18 mPas (cream soups) and 15.66-30.208 mPas (tarhana soups). Tarhana soups were more unique in consistency in comparison to tomato cream soups. The consistency of tarhana soups was thin with dissolved noodles, and because of that these samples had significantly lower viscosity in comparison to cream soups. Commercial tarhana soups had a consistency more similar to clear soups with noodles. Considering the literature data viscosity of commercial tarhana soup at 50°C was 32.33 mPas (Tahmaz et al. 2023) which is very similar to the value obtained for TT-3. Lower values for other tarhana soup samples (TT-1 and TT-2) are probably obtained because of higher measuring temperatures and different methods of determination. It should be mentioned that kinematic viscosity was measured by a capillary viscometer and dynamic (apparent viscosity) was calculated from values of kinematic viscosity and density at 55°C. In other studies (Hassan and Gadallah 2018; Ertop et al. 2019) higher viscosity values were reported for experimental tarhana soups (with fermented tarhana dough and the addition of different ingredients or processes to traditional recipes). Because of that fermented tarhana noodles have different physical and functional properties which consequently have an impact on final soup consistency and viscosity. Viscosity values of tomato cream soup samples varied in a bigger range than commercial tarhana soups, and the obtained results were in agreement with the literature. Chawan et al. (2018) reported that the viscosity of commercial tomato soups at 45°C ranged between 60 and 180 mPas, while Skotniska and Ocieczek (2019) reported values of 102-298 mPas for tomato soups. The viscosity of commercial tomato soup is about 40 mPas (USDA, 2008) According to producer specifications reported by Angelstarch (2024) viscosity values can range between 70 and 150 mPas. The obtained results are the most similar to data reported by the US FDA (2008), Chawan et al. (2015), and AngelStarch (2024).

Table 9 Results of physical properties of reconstituted ready to eat soup samples

Samples	Kinematic viscosity at 55°C (mm^2/s)	Apparent viscosity at 55°C (mPas)	Apparent fluidity $(Pas)^{-1}$	Adhesiveness (%)
TC-1	25.26±4-36^{c}	25.06±4.29^{c}	40.50±6-93^{ab}	0.27±0.08^{c}
TC-2	176.80±19.52^{a}	176.18±17.78^{a}	5.71±0.57^{c}	1.31±0.01^{a}
TC-3	132.09±5.94^{b}	131.07±6.42^{b}	7.64±0.37^{c}	0.63±0.13^{b}
TT-1	17.11±4.09^{c}	16.59±3.96^{c}	62.04±14.81^{a}	0.11±0.02^{c}
TT-2	15.73±0.01^{c}	15.66±0.10^{c}	63.86±0.41^{a}	0.13±0.02^{c}
TT-3	32,03±3.82^{c}	30.28±5.40^{c}	33.56±5.98^{b}	0.09±0-00^{c}

*Different small letters in columns represent statistically significant differences ($p \leq 0.05$) between samples

Adhesiveness (Table 9) of soup samples was measured at 5°C on liquid samples prepared according to producer, after 24h of cold storage. All soup samples showed expected very low adhesiveness, considering their liquid-thin consistency. Because of that adhesiveness was measured at a temperature of 5C, at which the samples had the highest density and viscosity with the thickest consistency. Adhesiveness ranged between 0.11 and 1.31%. Tomato cream soups had significantly higher consistency in comparison to tarhana soups. Sample TC-2 with the highest viscosity and density had the highest adhesiveness. This sample also had the highest amount concentration, rehydration ratio and reconstitution index. All these properties are closely related to each other, which also affects adhesiveness. Especially high solid concentration and high viscosity are the most relevant properties that could increase adhesiveness.

Table 10 Density of reconstituted ready to eat soups at different temperatures (kg/m^3)

Samples	100 °C	55 °C	25 °C	5 °C
TC-1	976.89±1.13^{C}	992.39±1.70^{B}	998.97±1.77^{B}	1008.83±2.68^{bA}
TC-2	957.89±37.67^{B}	997.01±9.47^{B}	1004.74±17.54^{B}	1340.00±38.18^{aA}
TC-3	951.60±26.86^{B}	992.16±4-02^{AB}	1007.23±1.74^{AB}	1050.09±36.65^{bA}
TT-1	985.33±1.43^{D}	969.65±0.21^{C}	1005.24±2.21^{B}	1012.93±1.51^{bA}
TT-2	918.61±101.40	995.85 at ±5.87	1015.50±0.71	1137.05±43.46^{ab}
TT-3	852.95±69.67	942.05±56.20	1010.74±1.32	1111.69±114.29^{b}

*Different small letters in columns represent statistically significant differences (p≤0.05) between samples, different large letters in rows represent significant differences between temperature

The density of soup samples varied (852.95-1340 kg/m^3) in dependence of temperature and sample type (Table 10). Density decreased in all soup samples when temperature increased. Differences in density between samples were more intense at low (5 and 25°C) than at high temperatures (55 and 100 °C). Significant differences between samples were obtained only at 5°C. The density of all samples was the highest at 5 C and the lowest at 100°C. The highest density values were obtained in the following samples: TT-1 at 100°C, TC-2 at 55°C, TT-2 at 25°C and TC-2 at 5°C. Density values were the lowest in the following samples: TT-3 at 100 and 55°C, and TC-1 at 25°C and 5°C. Differences between densities at different temperatures were significant in all cream soup samples and in only one tarhana soup sample (TT-1). TC-2 sample had the lowest moisture content, the highest viscosity and the highest values of density at 55°C and 5°C.

The obtained values were in agreement with the literature. According to the literature, data density varied in ranges 963-1017 kg/m^3 for tomato cream soups (Skotnicka and Ocieczek 2019, US FDA 2012) and 996.96 – 1050 kg/m3 for tarhana soups (Tahmaz et al. 2023, Celik et al. 2010). Differences occurred as a result of different testing temperatures and different compositions. Obtained density values (Table 10), which were higher and lower than the literature reported were noticed only at 100 and 5 °C, which was expected assuming that mentioned literature values are related to testing temperatures 20 – 55°C.

Thermophysical properties were estimated from moisture or total solid content in reconstructed ready-to-eat soup samples (Table 11). Samples with higher moisture content had higher values of all estimated thermophysical properties, which is in agreement with literature data (Singh and Heldman 2003). Estimated thermophysical properties varied in the following ranges: specific heat capacity above freezing point 3.88-4.05 kJ/kg K, thermal conductivity coefficient 0.554-0.592 W/mK, thermal diffusion coefficient 0.1420.146 mm2/s and freezing point between -1.77 and -0.60°C. As it was expected, the highest values for all thermophysical properties were noticed in the sample with the highest moisture content (TC-1), while the lowest was in the sample with the lowest moisture content (TC-2). There were no statistically significant differences. According to the literature specific heat capacity for similar products has the following values: 4.02 kJ/kg K for fresh tomatoes with 93% moisture, 3.676 kJ/kg/K for tomato concentrate with 81% moisture, and 4.18 kJ/kg K) for water, while thermal conductivity of fresh tomatoes was 0.528 W/(mK). The reported initial freezing point of fresh tomatoes with 93% of water content was -0.7 °C. Thermal conductivity, specific heat capacity, and thermal diffusion coefficient of water at 20°C have the following values: 0.599 W/mK, 4.18 kJ/kg K and 1.43 x 10^{-7} m^2/s (ASHRAE 2006; Singh and Heldman 2009, Toledo 1994). The estimated freezing point for soup samples with 84.85-94.52% of moisture had very similar or slightly lower values in comparison to reported. Lower values of freezing point are related to higher total solids. It is also important to mention that the Guegovs model is suitable for the freezing point of a very huge group of liquid foods with moisture content between 73 and 96.5%. All soup samples had moisture content in the mentioned range. Thermal conductivity was estimated by a model reported by Dickerson which is suitable for food products with water content higher than 50%. Lower values obtained for specific heat capacity and thermal conductivity in comparison to literature could be explained by lower moisture content in samples. Thermal diffusion coefficient also increased with higher moisture content.

Table 11 Results of thermophysical properties

Samples	Specific heat capacity (kJ/kgK)	Thermal conductivity (W/mK)	Thermal diffusion (mm^2/s)	Freezing point (°C)
TC-1	4.05±0.01	0.592±0.00	0.146±0.00	-0.60±0.07
TC-2	3.88±0.21	0.554±0.05	0.142±0.00	-1.77±1.47
TC-3	4.02±0.01	0.585±0.00	0.145±0.00	-0.80±0.04
TT-1	3.96±0.01	0.572±0.00	0.144±0.00	-1.20±0.05
TT-2	4.01±0.05	0.582±0.01	0,143±0.00	-0.90±0.27
TT-3	4.04±0.01	0.589±0.01	0.144±0.00	-0.68±0.20

CONCLUSION

In comparison to tomato cream soup powders, tarhana soup powders had higher large particle amounts (>1mm), higher bulk and tapped density, lower foam capacity, hygroscopicity, angle of repose, Hausner ratio, and Carr index. Tarhana soup powders had higher flowability and lower cohesiveness in comparison to cream soups. According to the Hausner ratio and Carr index flowability of tarhana soup powders was assessed as fair to passable, while tomato cream soups were poor to extremely poor. Considering the angle of repose values flowability was assessed as fair to passable (need to be hung to flow) for cream soup powders and as fair (aid not need to flow) for tarhana. Samples with higher noodle content had lower dispersibility, lower angle of repose, and better flowability. The cohesiveness of soup powders was low to normal, higher in cream soups. According to the angle of repose values, all samples had low cohesiveness. Because of that Hausner ratio and Carr index should be used as better flowability indicators than the angle of repose. Cream soup powders had a thicker consistency, higher rehydration ratio, higher amount of small particles (<0.5 mm), higher viscosity, foam capacity and adhesiveness, lower bulk and tapped density and lower powder flowability in comparison to tarhana soups. The obtained results were in agreement with the literature data. Commercial tarahana soup samples were prepared from nonfermented pasta dough and because of that some properties differed from the literature. The density of reconstituted samples increased when temperature decreased, while thermophysical properties increased with moisture content.

REFERENCES

Abd-Elhak N.A., Salem A.A. (2017). Preparation and evaluation of of dried soup produced from different sweet potato varieties. Egypt. J. Agric. Res. 95(3), 1161-1177

Abdel-Haleeem A. M. H., Omran A. A. (2014). Preparation of Dried Vegetarian Soup Supplemented with Some Legumes, Food and Nutrition Sciences, 2014, 5, 2274-2285.

Alawode E.K, Idowu M.A., Adeola A.A., Oke E.K., Omaniyi S.A. (2017). Some quality attributes of complementary food produced from flour blends of orange fresh potato sorghum and soybean, Croat. J. Food Sci. Technol. 9 (2), 122-129

Ali, Md. Y., Ibn Sina, A.A., Khandker, S.S., Neesa, Tanvir, E.M., Kabir, A., Khalil, Md. I., S.H. Gen, 2021. Nutritional composition and bioactive compounds in tomatoes and their impact on human health and disease: A review. Food Process Engineering, Foods, 10, 45.

Angelstarch (2024). Instant tomato soup mix powder (https://angelstarch.com/instant-tomato-soup-mix-powder/)

Ansari F., Singh A., Baidya K., Rana G.K, Bharti A. (2020). Formulation and development of instant soup mix using Moringa oleifera leaf powder. Journal of Pharmacognosi and Phytochemistry, International Web-Conference on New Trends in Agriculture, Environmental & Biological Sciences for Inclusive Development 21-22 June 2020, 429-432

AOAC (1995). Official methods of analysis 16th Ed. Association of official analytical chemists. Washington DC, USA.

ASHRAE, 2006. Chapter 9, Thermal properties of food. (In: Handbook Refridgeration.).

Asma M.A., El Fadil B., El Tinay A. H. (2006). Development of weaning food from sorghum supplemented with legumes and oil seeds, Food and Nutrition Bulletin 27(1) 26-34

Bader S. J., El–sayed S. M., Galila, Asker G.A.H. (2022). Quality Analysis of Development Dried Vegetable Soup fortified with Purslane Leaves and Seeds powder, New Valley Journal of Agricultural Science NVJAS. 2 (6) 2022, 372-383.

Bhargavanandha K.S., Kshirsagar R.B., Patil B.M., Bachate A.H. (2021). Effect of addition of partially hydrolyzed guar gum on the quality attributes of tomato soup powder. The Pharma Innovation Journal 10(9), 627-634 .

Cagindi O., Z. Aksoylu, N. Y. Savlak, Kose E., 2016. Comparison of physicochemical and functional properties of domestic and commercial tarhana in Turkey. Bulg. J. Agric. Sci., 22: 324–330

Celik I., Isik F., Yilmaz Y.: Chemical, rheological and sensory properties of tarhana with wheat bran as a functional ingredient. Akademik Gida 8(3), 11-17 (2010).

Chawan R., A., Basu S., Nema P. K., Nalawade T. (2015). Whey Based Tomato Soup Powder: Rheological And Color Properties, International Journal of Agricultural Science and Research (IJASR), Vol. 5, Issue 4, Aug 2015, 301-314

Dağtekin, B.B., Balçık Mısır, G. (2023). A Preliminary Study on The Use of Protein Isolate Produced From Trout in Tarhana Soup. Aquatic Food Studies, 3(1), AFS182. https://doi.org/10.4194/AFS182

Delgado, A.E., Sun, D.W. & A.C. Rubiolo, 2006. Thermal Properties of Food. (From: Thermal Food Processing – New Technologies and Quality Issues. Ed. Sun, D.W., Francis Taylor Group.

El-Gindy A.A. (2018).Preparation of some weaning food formulated from sweet potato, millet and quinoa, Current Science International ISSN: 2077-4435, 7(4) 662-679.

Elrih W.M., Ismael I.A. (2020). Development of quick dried soup powder mixes and easy to prepare. International Journal of Multidisciplinary Research and Publications 3(3), 91-94

Ertop M.H., Cerit Z.G., Atasoy R. (20119). Evaluation of Physicochemical, Nutritional and Sensory Properties of the Wet Tarhana, Food Science and Quality Management Vol. 83, 61-67.

Fernandes, R. V. de B., Borges, S. V., & Botrel, D.A. (2013). Influence of spray drying operating conditions on microencapsulated rosemary essential oil properties. Ciência e Tecnologia de Alimentos, 33, 171–178.

Fernandez-Lopez J., Bottela-Martinez C., de Vera C. NR., Sayas-Barbera M.E., Viuda-Martos M., Sanchez-Zapata E., Perez-Alvarez J.A. (2020). Vegetable soups and creams: Raw materials, processing, health benefits and innovation trends, Plants 2020 (9), 1-33

Gandhi N., Singh B., Sharma S. (2017). Storage stability and quality assessment of instant vegetable soup mixes prepared by extrusion processing. Bulletin on Enviroment, Pharmacology and Life Sciences 6(6), 73-82

Gawad G.S., Shelke R.R., Jadhao K.V., Kahate P.A., Nage S.P., Bidwe K.U. 2022. Effect of tomato powder on Physico-chemical properties of Paneer Whey Soup, The Pharma Innovation Journal; 11(2): 2590-2592.

Gogari S. (2022). The Effect of Quinoa and Chickpeas Flours on the Physical, Chemical, Functional and Sensorial properties of Tarhana Soup, Egypt Journal for Specialized Studies - Vol (10) N (35), 5-28.

Goncu A., Celik I. (2020). Investigation of some properties of gluten-free tarhanas produced by red, green and yellow lentil whole flour, Food Sci. Technol, Campinas, 40(Suppl. 2): 574-581.

Gould, W.A., (1992). Tomato Production, Processing & Technology – Third edition. CTI Publishing Inc.

Guegov, J., 1980. Journal of Food Technology, 15. 607.

Hassan M. F. Y., Gadallah M.G.E. (2018). Physico-chemical and Sensory Properties of Tarhana Prepared from Different Cereals and Dairy Ingredients, Current Journal of Applied Science and Technology 29(3): 1-14, 2018; Article no.CJAST.38459.

Hlavač, P., Božikova, M. & Z. Hlavačova, (2019.) Selected properties of some tomato ketchups. 7th TAE 17-20 September 2019, Prague Czech Republic, 165-170

Imtiaz H., Burhan Uddin, M., Gulzar M.A. (2007). Evaluation of weaning foods formulated from germinated wheat and mungbean from Bangladesh. African Journal of Food Science, 5 (17) 897-903

Jamshidvand M., Gallan N., Dermiki M. (2023). Development of High Protein Tomato Soup Using Milk Protein Concentrate and Pea Protein Isolate

Jan S., Rafiq S. I., Saxena D.C (2015). Effect of Physical Properties on Flow ability of Commercial Rice Flour/Powder for Effective Bulk Handling. International Journal of Computer Applications (0975 – 8887),

Kambabazi M-R., Okoth M.W., Ngala S., Njue L., Vasanthakaalam H. (2022). Physicochemical properties and sensory evaluation of a bean-based composite soup flour, legume Science 4 (15).

Kamble K.S., Mote G.V., Sahoo A.K. (2019). Process development for instant Moringa pod soup powder supplemented with herbs. Journal of Pharmacognosy and Phytochemistry 8 (3), 3281-3286.

Koc G. C., Ozcira N. (2019). Chemical composition, functional, powder, and sensory properties of tarhana enriched with wheat germ, J Food Sci Technol. 56 (12): 5204–5213

Kour K., Sood M., Bandral J.D., Gupta N., Kumari A. (2024). Physical, Functional and Microbiological Parameters of Tomato-Broccoli Blended Instant Vegetable Soup Mix, European Journal of Nutrition & Food Safety Volume 16, Issue 4, Page 102-109, 2024

Kumari A., Kaushik N., Slizyte R., Khusboo (2023). Formulation of a Ready-to-Cook Soup Mix Using Microencapsulated Protein Hydrolysate Obtained from Pink Perch By-Product, Polish Journal of Food and Nutrition Sciences Vol. 73, No. 3, 253–264.

Lakišić A. (1999). Bosanski kuhar – Tradicionalno kulinarstvo u Bosni i Hercegovini, 6. Izd. Svjetlost Sarajevo.

Mohajan S., Orchy T.N., Farzana T. (2018). Effect of incorporation of soy flour on functional, nutritional and sensory properties of mushroom-moringa-supplemented healthy soup. Food Science & Nutrition published by Wiley Periodicals Inc., 1-8

Ocieczek A., Palich P. (2007).The influence of development of the specific surface of sorption on the wettability of instant soups. Czes. J. Food Sci. 25(6), 333-338.

Ramadan, M.A. Khalil, K. I., El Robi, M.H. & A.S. Othman, (2021). Quality characteristics of Tomato Fruits for processing based on chemical composition and functional compounds in response to fertilization rate with potassium humate. Fayoum Journal of Agricultural Research and Development FJARD, 35 (2): 245-258.

Sahni S., Shere D.M .(2017). Comaparative evaluation of physico-chemical and and functional properties of apple, carrot and beetroot pomace powders, Int J. Food Ferment Technol. 7(2), 317-323.

Sihsobhon, et al.(2013). Physicochemical Properties and Sensory Evaluation of the Formulated Reduced Calorie Satay Sauce, Kmitil Science and Technology Journal 13 (2).

Singh P.R., Heldman D.R. (2003). Introduction to food process engineering, 3rd Edition. Academic Press. Food science and technology, International series.

Singh V.,Kaur K. (2020). Development, formulation and shelf life evaluation of baby corn soup mix from industrial by-products, Journal of Food Science and Technology Vol. 57, 1917–1925.

Skotnicka M., Ocieczek A. (2019) .nfluence of tomato cream soup physical parameters on satiety potential level. Acta Agroph. 26(4), 5-21

SSepuuya G., Katongole J., Tumuhimbise G.A. (2018). Contribution of instant amaranth (Amaranthus hypochondriacus L.) - based vegetable soup to nourishment of boarding school adolescent. Food Science&Nutrition, Wiley Periodicals Inc., 1402-1409

Szulz K., Lenart A (2016) Effect of composition on physical properties of food powders, Inter-national Agrophysics 30, 237-243 doi. 10.1515/intag-2015-0084.

Tahmaz J., Somun I., Operta S., Oras A., Alkić-Subašić M., Karahmet E. (2023). Quality of Commercial Blends for Tarhana,Bey’s and Sarajevska Soup, M. Brka et al. (Eds.): Agriconference 2022, LNBE, Springer Nature Switzerland AG 2023 pp. 285–302

Tarkci Z., Dogan I.S., Koca A. F. (2004).. A traditional fermented Turkish soup, tarhana, formulated with corn flour and whey. Int. J. Food Science and Technology 39, 455 – 458

Toledo, R. T., 1999. Fundamentals of Food process engineering, 4th edition, AP Elsevier.

US FDA/CFSAN: (2008). Acidified and low acid canned foods – Approximate pH of foods and food products. 1-13

Verma A., Mogra R. (2017). Process for the preparation of value addedtomato-mushroom soup mix incorporated with Psillium husk and its quality evaluation. International Journal of Pure & Applied Bioscience 5 (5), 1502-1507

Yadav Y.S., Prasad K., Kumar S. (2022). Ingredients interactions effect on development and characterization of rice flour and Moringa oleifera leaf powder based instant soup mix., Chemical Science Review and Letters 11 (42), 111-122.

THE INFLUENCE OF FERTILIZATION ON THE MORPHOLOGICAL CHARACTERISTICS, YIELD AND QUALITY OF SOME SWEET POTATO VARIETIES

Lidija MILENKOVIĆ[1], Zoran S. ILIĆ[1], Ljubomir ŠUNIĆ[1], Dragana LALEVIĆ[1], Žarko KEVREŠAN[2]

[1]University of Priština in Kosovska Mitrovica, Faculty of Agriculture - Lešak, Field and Vegetable Crops Department, Serbia
[2]University of Novi Sad, Institute of Food Technology, Novi Sad, Serbia

Corresponding author e-mail: lidija.milenkovic@pr.ac.rs

ABSTRACT

Although sweet potato is almost the most important crop in many Asian, African and Latin American countries, there are only sporadic research data on production in some continental European countries (Western Balkan countries). Therefore, this research presents an overview of the morphological traits, yield and sensory properties of three phylogenetically different sweet potato cultivars: Beauregard (orange flesh), Japanese (light flesh) and 414 (purple flesh) under several fertilization regimes: 1. poultry pellet fertilizer – control; 2. $NP_2O_5K_2O$ 100:100:100; 3. NPO_5K_2O 100:100:100 + 50N. The mentioned varieties have thin stems with a strong habitus, which cover more than 50% of the inter-row spacing by the 40th day of vegetation. Morphological characteristics of the plants: the length of the main branch, the length and number of side branches, the number of leaves varies during the growing season regardless of the treatment. However, the increased dose of nitrogen (150N) contributed to a higher total leaf mass measured immediately before harvesting the tubers in all three varieties (1476.7 -2470.0 g/plant) compared to other treatments (1300.7-2359.3 g/plant). Treatment with an increased dose of nitrogen (150 N) had a positive effect on the growth of leaf mass in all three varieties. However, a higher dose of nitrogen only in variety 414 affected the increase in tuber yield (25.2 t • ha $^{-1}$) compared to other treatments. For Japanese (60.2 t • ha $^{-1}$) and Beauregard (38.9 t • ha^{-1}), increased nitrogen rates contributed to a decrease in total yield compared to the fertilization treatment with uniform NPK rates (66.6 t • ha $^{-1}$; 42.6 t • ha $^{-1}$). Poultry manure is not able to satisfy the plants needs for nutrients, so the tuber yields of all three varieties in this treatment are statistically lower. Regardless of the fertilizer treatment, the sensory properties of tubers depend on the characteristics of the variety. The marketable yield of Japanese (82.0-89.8%) and Beauregard (79.3-88.1%) is a quite uniform. The mentioned varieties have a significantly higher market yield compared to 414 (54.1-70.8%) and the reason is the proportion of small and tail roots, as well as tuber deformities. Based on the tasting panel, Beauregard received the highest rating, given that it is a commercial variety and is well known to consumers. After cooking, they have a soft consistency, with less fiber and veins, a pleasant aroma and a sweet taste, reminiscent of pumpkin. Japanese and 414 are less readily available for sale (mainly at local farms and markets), suggesting that advertising and tasting should attract consumers.

Keywords: Sweet potato, Variety, Fertilizer, Morphological characteristic, Yield

INTRODUCTION

Specific nutrients and health-promoting values in food crops are of great interest. Sweet potato (*Ipomoea batatas (*L.) Lam.) is an important food, livestock, industrial and energy crop yielding about 200 million metric tons annually on 9 million hectares (Yu et al., 2020). Edible leaves and tubers provide carbohydrates, fiber, carotene, B vitamins, vitamin A and C (El Sheikha and Rai, 2017), and some functional ingredients such as chlorogenic acid which provide medicinal value through antioxidant, antimicrobial, anti-inflammatory effects (Xu et al., 2012; Liang and Kitts, 2016). Epidemiological studies suggest that regular consumption of plant-based foods plays a key role in the prevention of chronic diseases, such as cancer, diabetes, cardiovascular disease and neurodegenerative diseases. Sweet potatoes are considered low on the glycemic index scale and are categorized as one of the powerful functional foods due to their high content of dietary fiber and phenolics (Boeing et al., 2012). Sweet potato is among the major food crops in the world and is grown in all tropical and subtropical regions, especially in Asia, Africa and the Pacific. The regions of Asia and Africa account for 95% of world production. However, in Serbia and neighboring countries, such as Croatia, Hungary and Romania, sweet potato is a relatively new crop, which is mainly grown on individual farms and small gardens. Intensive production has been noticeable in recent years, and consumers are slowly starting to introduce sweet potato root into their diet (Milenković et al., 2024). Climate change is evident. During the summer of 2024 in Serbia was recorded an increased number of tropical days (t above 30.0C) and tropical nights (t above 20.0C). Years ago the presence of late summer was noticeable (RHMZ), which is the basis for the development of new strategies in agriculture. We are of the opinion that sweet potatoes can occupy a significant place in the economy of our region. Sweet potato is a starchy crop with high potential to meet food security and nutrition needs for a sustainable society in the twenty-first century. Due to its high nutritional content and wide adaptability to marginal lands in areas ranging from tropical to temperate zones, sweet potato is a promising crop to prevent malnutrition and increase food security in developing countries (Kwak, 2019). Farmers from rural areas prefer sweet potato cultivation because it is a rustic, easy-to-maintain and drought-resistant crop with relatively low production costs, minimal inputs and high yields per unit area (Volmann et al., 2021). However, in some countries, low yields are recorded, which are related to the unavailability of nutrients (Nsa et al., 2013; Fernandes et al., 2021). Therefore, Udo et al. (2005) state that the availability of nutrients significantly contributes to a good crop yield and note a significantly lower yield of the tested when no fertilizer was applied, as a consequence of the poor availability of nutrients in the soil, which is common in traditional agricultural systems. By applying appropriate management techniques that should be developed at the local level, higher crop yields can be achieved. It is also necessary to choose varieties whose quantitative and qualitative properties correspond to the needs of the market.

The purpose of this research is to examine the performance of some sweet potato varieties in different feeding regimes in order to achieve a higher total and market yield.

MATERIAL AND METHOD

Sweet potato production was done in a random block system with three repetitions, during the year 2023, on an experimental plot in the village of Moravac near Aleksinac (21°42′, 43°30′, altitude 159 m), on soil with favorable physical and mechanical properties, alluvium type (Table 1). The examination included three varieties: Japanese (light flesh), Beauregard (orange flesh) and 414 (purple flesh) in three feeding regimes: 1. poultry pellet fertilizer – control; 2. $NP_2O_5K_2O$ 100:100:100; 3. $NP_2O_5K_2O$ 100:100:100 + 50N. As nitrogen content is somewhat lower (0.18%), we paid more attention to this element and increased nitrogen doses in the fertilization treatment. A randomized block design with two factors with three replications was employed. The first factor was the genotypes of sweet potato. Three cultivars of SP with different flesh and peel color potatoes ('Japanese'mid-early cultivar with pink skin-white fleshed; 'Beauregard' mid-early cultivar with orange skin-orange fleshed and '414' late cultivar with purple skin purple-fleshed) were provided by company from the east part in Croatia (cadastral area of Ilok) and were included in experiment. The second factor was different plant nutrition; organic (as control) and inorganic fertilized as treatments. Organic poultry manure were used with 900 kg/ha granulate (Italpollina with $N:P_2O_5:K_2O$ 4:4:4, organic carbon 35%, ratio C/N 8/8) and two inorganic fertilizers, first treatment - mineral complex $N:P_2O_5:K_2O$ 100:100:100 and second treatment – $N:P_2O_5:K_2O$ 100:100:100 + 50N was applied. Complex fertilizer of the combination 16:16:16 was used as source of $N:P_2O_5:K_2O$. As a source of additional N fertilizers was used KAN (27%) from the company Genezis. Production is based on using seedlings that at the moment of planting had formed 5-6 leaves. Planting was done on May 20, 2023, with a distance between rows of 120 cm and in a row of 35 cm, with 2.4 plant on. m^{-2}, on banks mulched with PE-foil with a drip irrigation system. The basic plot included 15 plants. Morphological characteristics: length of main and side branches (cm), number of lateral branch, number of leaves, shape of leaves, color, followed are in samples of five plants randomly selected from each plot, 40 and 60 days after planting, based on the descriptors of Huaman (1991). Collection of thickened roots was done 110-120 days after planting when total yield (t ha-1), market yield , as well as yield components: mass of roots per plant (g), number of roots per plant, average root weight (g), length (cm) and width (cm). After harvesting, the taste was evaluated by cooking the samples. Quantitative descriptive analysis was applied to evaluate the samples in terms of the aroma, texture, flavor and aftertaste attributes (Tomlins et al., 2003). The obtained results were statistically processed by analysis of variance and LSD test (MSTAT-C).

Table 1. Chemical analisys of soil (0-30cm depth)

pH in 1M KCl	pH in H_2O	$CaCO_3$ %	Humus %	N %	P_2O_5	K_2O
					mg/100g	
6.62	7.54	0.69	3.59	0.18	40	40

RESULTS AND DISCUSSION

Sweet potato (*Ipomoea batatas* L. Lam., 2n=6k=90) has become an accessible source of calories, protein, fiber, minerals, vitamins and flavonoids (Padmaja, 2009), especially in countries in development. As the tuberous root of the plant is most commonly consumed in the western part of the world, leaves are increasingly of interest for human consumption due to their nutritional value (Suarez et al. 2020). Namely, the leaves are a good source of protein, fiber, polyphenols and minerals, and the polyphenols in them are important antioxidants. Also, it was reported that the polyphenol content of the leaves was much higher than that of the whole root, fleshy tissue and skin of sweet potato, as well as the second largest commercial vegetable (Truong et al., 2018). Villareal et al., (1982) indicate that sweet potato leaves are consumed as a vegetable in tropical areas, especially in Southeast Asia, and Sun et al. (2014) suggested that green mass yields are significantly higher than leafy vegetables as an off-season alternative. Since the plants grow quickly in wet conditions, these leaves can be harvested several times a year without adversely affecting the tuber yield. In some communities in Nigeria, Brazil, the leaves are used fresh, heat-treated, dried as a supplement to flour. The yield of leaves in certain varieties can be higher compared to the root, which is also a feature of the described variety 414 in our research.

Although the phylogenetically different cultivars Japanese and Beauregard on the 60th day of vegetation achieve a uniform length of the main branch, a uniform number of side branches, and these values are significantly lower compared to variety 414. Sweet potato is a herbaceous liana plant with alternating leaves and tubular flowers. The leaves are smooth, 10-15 cm lenght and 10-15 cm widht. The shape of the leaves can vary even on the same plant, but basically they are round whole to deeply incised triangular leaves, with slight lobes in 414, smooth edge and smooth surface, quite firm consistency. In variety 414, the veins are reticulate, medium to strongly pronounced. The leaves of the Japanese variety are of a delicate consistency, with a smooth edge and surface, with a moderate nervature, which can potentially be considered a desirable feature as a leafy vegetable (personal observation). Milenković et al. (2023) made a similar conclusion for the O Henry light type variety. Young leaves can be consumed (Antonio et al., 2011). The basic leaf color is green in Beauregard and 414, and lighter green in Japanese. Purple as a secondary color (according to Human, 1991) is observed on the veins and edges of the leaves in all three varieties, but it is most intense in the variety 414. The above-mentioned varieties have mainly prostrate stems with a strong habit, which cover more than 50% of the inter-row spacing by the 40th day of vegetation. The soil coverage with vegetative mass is excellent (Table 2), and branch nodes in contact with the soil develop adventitious roots, resist weeds and reduce soil erosion in case of heavy rainfall. Leaf shape and color, petiole length, and leaf spacing mey make this species useful in ornamental arrangements as well as in eroded landscaping (Loebenstein et al., 2003).

Differences in plant leaf mass between cultivars are very significant, while the difference between types of fertilization was significant (Table 3).The growth of shoots and tubers of plants showed a different response to N availability. N deficiency inhibits vine growth much more than crossgrowth. The differences between the varieties are very significant, so the 414 variety compared to the other two produces a significantly lower yield of tubers, and at the same time gives the highest leaf mass compared to the other two varieties in all variants. Morphological characteristics of the plants: the length of the main branch, the length and number of side branches, the number of leaves varies during the growing season regardless of the treatment, however, the increased dose of nitrogen (150N) contributed to a higher total leaf mass measured immediately before harvesting the tubers in all three varieties (1476, 7 -2470.0 g/plant) compared to other treatments (1300.7-2359.3 g/plant)(Table 3). Varieties react differently to organic fertilization. All morphological parameters vary in the period from 40 to 60 days after planting. The number of leaves on the 60th day after planting in Beauregard was uniform regardless of the treatment. Mineral fertilizers significantly increase the number of leaves at 414. The increased number of leaves of the variety Japanese was initiated by a uniform dose of NPK. The position of the branches on the plant also affects the number of leaves. Increased doses of nitrogen (N150) contributed to a significantly higher yield of leaf mass compared to other nutrient treatments. Nitrogen fed at an early stage of crop development will help build the overall size of the leaf canopy, whereas at later stage of growth, nitrogen helps maintain the greenness of the canopy and maximize yield (Mark et al., 1983).

Considering that there are no significant differences between the fertilizer treatments in 414, the intensive growth of the vegetative mass can be considered a variety al trait. In contrast to this, the Japanese variety formed a significantly higher leaf mass when applying a higher dose of nitrogen compared to the other fertilization treatments. Both mineral fertilizer treatments affected the achievement of uniform leaf mass in the cv.Beauregard compared to the chicken manure treatment. Many studies indicate a positive effect of organic fertilizer application on the yield and quality of sweet potatoes. However, our research showed that the application of chicken manure could not meet the nutrient needs of sweet potatoes compared to mineral nutrients. Basically, organic matter is a key component of soils that affects their physical and chemical properties such as water retention, erodibility, cation exchange capacity and nutrient availability (Rice, 2002; Deksissa et al., 2008). Maintenance of soil organic matter levels and optimization nutrient cycling is essential for the sustainable productivity of agricultural systems (Aiuke et al., 2004; Khan et al., 2013).

Sweet potato leaves are an excellent source of chlorophyll and carotene. Dinu et al. (2021) suggest that the higher content of dry matter contributes to the greater nutrition of sweet potato leaves compared to some other vegetable species, and they should be considered for human consumption.

Villordon et al. (2009), indicate that about 90% of the tuber develops from the first adventitious roots formed immediately after planting. However, unfavorable conditions can cause many or all of these to develop into primary fibrous roots or to lignify and produce pencil roots. Minimizing stresses such as high nitrogen levels, low oxygen or dry conditions impacts many of the cultural practices for sweet potato in highly developed production systems (Troung, 2018). Nitrogen, phosphorus and potassium are among the essential elements required for plant metabolism and improving soil water holding capacity (Wamba et al., 2012). Nitrogen is largely needed during leaf formation and then for increasing tuber growth and size, when it ensures optimal photosynthate production in the leaves (Taffouo, 1994).

Varieties Japanese and Beauregard have a uniform average mass of tubers, regardless of the method of fertilization (Table 4). The variety and method of fertilization do not influence the average number of tubers. The differences in yield between the cultivars are significant, so cultivar 414 compared to the other two achieves a significantly lower yield of tubers..Treatment with a dose of nitrogen 150N contributed to an increase in total yield only in variety 414. Higher mineral N rate increased growth of sweet potato vines (Fernandes et al., 2018). In the case of other varieties, the treatment with uniform mineral nutrients had an effect on the increase of the total yield, but without a statistically significant difference compared to the application of 150N. N fertilization affects the development of sweet potato storage roots and can increase their protein content Ca and Mg. However, N fertilizers are expensive for farmers, and high N rates can reduce starch content in sweet potato roots, increase fibrous root formation, reduce storage growth roots, and increase the yield of unmarketable storage roots (Fernandes et al. 2021).. In our research, we observed the presence of lignified pencil roots in clusters of tubers, which is in accordance with previous statements.

Although in 414 plants achieve a uniform leaf mass when treated with organic and inorganic fertilizers, the mass of tubers is significantly lower when chicken manure is applied. The same treatment contributed to the reduction of the commercial yield of this variety by more than 40%, which is a significantly higher value compared to the other treatments. Commercial yield differences between Japanese and Beauregard cultivars are evident but not significant. In our research, the commercial yield is a part of the total yield that is reduced by the mass of fibrous root formation (pencil root), deformed, damaged tubers by mechanical or soil pests, as well as tubers weighing less than 100g (Chart 1, Chart 2). Fertilization with chicken manure contributed to the highest proportion of non-commercial roots weighing less than 100g in 414, unlike other varieties that have a uniform average weight (within the commercial range) regardless of the method of fertilization. Damage by soil pests was assessed based on the International Potato Center-CIP scale (Gruneberg et al., 2010) and all three cultivars showed some damage in the range of 2-4: minor to moderate (data not shown).

Different authors use different criteria to distinguish commercial roots from non-commercial roots. According to Filgueira (2008), the sweet potato is considered commercial when it presents roots with average mass ranging from 200 g to 400 g. This classification is different from study to Selmane et al.(2024) which suggest that roots weighing less than 100g belong to the non-commercial class. The Japanese variety, as well as most light-type varieties, are high yielding with roots weighing over 500 g. Although large roots (over 1000g) are categorized as non-marketable, consumers prefer to accept them compared to small ones (less than 100g), and they can also find a place in industrial processing. The differences in commercial root yield could be attributed to the genetic variations among the sweet potato varieties in partitioning photosynthesis (Nedunchezhiyan et al.,2007). According to Pedrosa et al. (2010) the total productivity of roots is dependent on the genotype, soil and climate conditions, and cycle duration. Variety 414 is late growing. It is assumed that by changing the date of planting and the method of production (greenhouse production), this variety would improve its genetic potential by increasing the total and commercial yield. The benefits of purple pigmentation varieties have been confirmed in several studies due to the capacity (property) of anthocyanins to provide multiple benefits to the human body. Because 414 forms a longer and thinner tuber compared to Beauregard and Japanese, it is prone to damage at harvest. However, one must be careful during harvesting to avoid skinning or bruising the produce. Skinning and bruising can spoil the roots, making them unappealing to consumers. Farmers must make every effort to minimize mechanical damage, ensuring an appropriate shelf life and acceptance of the product (Todesco et al., 2023).

Important characteristics of the varieties are: resistance to drought, high content of dry matter, resistance to diseases and pests. The color of sweet potato skin ranges from white, cream, yellow, orange, pink, and red to purple, while flesh colors may be white or various shades of cream, yellow, orange, or even purple (Teow et al., 2007; Alam et al., 2016). In Serbia the orange pulp variety is the most common. The sweetness of the root is considered an important flavor attribute. It can also be a complex characteristic, not necessarily directly related to sugar content. The sugar content of the root increases during cooking, as the process promotes the breakdown of starch. Beauregard possesses sweetness (Chart 4).The texture of the thermal processed tuber is an important attribute on which consumer’s acceptance of the tested varieties depends. In Beauregard, the texture is soft, spreadable, moist and denser (pasty) compared to other varieties tested. The pulp is homogeneous, without secondary color diffusion. A less sweet Japanese variety (chart 4). Grainy, moist texture is identified. The taste is neutral, without an aftertaste. In 414, the sweetness is moderate, it is a bit astringent, with a slight earthy after taste. The dietary fiber content of sweet potato roots varies between different cultivars. The proportion of fibers was evaluated in the following order: the highest in variety 414, then Japanese and then Beauregard. Our research has shown that 414 has a significantly higher fiber content compared to Japanese and Beauregard. Leighton et al. (2010) suggested that the Blesbok variety VFSP (white pulp) has a higher fiber content than the variety OFSP (orange pulp). In some varieties, the fiber content can be so high that sweet potatoes are unusable directly as human food and are directed towards industrial processing.

Table 2. Morphological characteristics of the sweet potato plants

Cultivar	Type of plants	Predominant color of branches	Color of the mature leaves	Color of the immature leaves	Color of petiole; length	Main flesh (pulp) color
Beauregard	Spreading; prostrate	Grin / sporadically purple the top of the branches	Grin	Grin with purple edge	Grin with purple nodes; short (10-20cm)	Orange flesh
Japanese	Spreading; prostrate	Grin/ sporadically light purple the top of the branches	Grin	Grin with liht purple edge	Grin with purple nodes(sporadically); Short (10-20cm)	Cream flesh
414	Spreading-exstreamly spreading; prostrate	Grin / purple the top of the branches	Grin/purple	Grin/purple	Grin/purple Short/Intermediate(15-25cm)	Intense purple

Table 3. Effect of fertilization on morphological characteristics of sweet potato plants

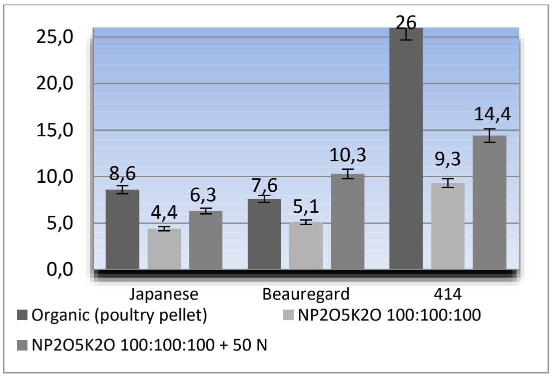
Cultivar	Type of fertilization	40 days after planting				60 days after planting				Plant mass before harvesting
		The length of the main branch (cm)	The number of the side branches	The length of the side brunches(cm)	The total number of leaves	The length of the main branch (cm)	The number of the side branches	The length of the side brunches (cm)	The total number of leaves	Plant mass (g /plant)
Japanese	Organic (poultry pellet)	55.8 cd*,cde**	5.37bc,b	36.4b,b	76.8c,cde	104.95c,bc	7.1b,c	72.6b,b	138.1bc,b	1420.0 e,cd
	$NP_2O_5K_2O$ 100:100:100	65.6b,abc	5.6bc,b	44.6a,a	106.9a,ab	106.3 c,bc	7.5b,c	77.2b,b	168.6a,ab	1495.0 de,cd
	$NP_2O_5K_2O$ 100:100:100 + 50 N	62.3bc,bcd	5.7b,b	43.07a,a	80.4bc,bcd	100.5c,c	7.2b,c	74.3b,b	147.4bc,ab	1793,3 cd,bc
Beauregard	Organic (poultry pellet)	45.7e,e	3.8d,c	26.07c,b	49.7d,e	108.6bc,bc	7.1b,c	70.1b,b	131.8c,b	1300.7 e,d
	$NP_2O_5K_2O$ 100:100:100	52.1de,de	4.7cd,bc	27.5c,c	61.8cd,de	115.5bc,abc	7.4b,c	78.4b,b	135.4bc,b	1406.0 e,cd
	$NP_2O_5K_2O$ 100:100:100 + 50 N	62.3bc,bcd	4.6cd,bc	26.5c,c	61.4cd,de	126.6ab,ab	7.9b,bc	74.3b,b	136.7bc,b	1476.7 de,cd
414	Organic (poultry pellet)	68.43ab,ab	8.5a,a	33.14b,b	97.9ab,abc	126.53ab,ab	9.4a,a	105.7aa	165.26abc, ab	2094.0bc,ab
	$NP_2O_5K_2O$ 100:100:100	76.0a,a	8.4a,a	34.17b,b	107.5a,ab	140.6a,a	9.5a,a	116.7aa	190.7a,a	2359.3 ab,a
	$NP_2O_5K_2O$ 100:100:100 + 50 N	76.13a,a	8.6a,a	36.21b,b	110.7a,a	138.7a,a	9.7a,a	118.8a,a	193.1a,a	2470.0a,a

*Different letters indicate a significant difference as determined by ANOVA, *factor significance < 0.05; factor significance ** <0.01.

Table 4. Effect of fertilization on yield of sweet potato varieties

Cultivar	Type of fertilization	Average mass of tuber (g)	The number of tuber per plant	The length of tuber (cm)	The widht of tuber (cm)	Total yield of tuber (t • ha $^{-1}$)	Marketable yield (%)	Marketable yield (t • ha $^{-1}$)	Unmarketable yield (t • ha $^{-1}$)
Japanese	Organic (poultry pellet)	575.5a*, a**	4.2bc, a	22.43a,a	7.50ab,ab	53.1bc,ab	82.0	43.6	9.55
	$NP_2O_5K_2O$ 100:100:100	527.6a,a	5.5ab, a	21.50ab, ab	7.67ab,a	66.6a,a	89.8	59.78	6.8
	$NP_2O_5K_2O$ 100:100:100 + 50 N	569.8a,a	4.7abc ,a	22.36a,a	8.03aa	60.2ab,a	87.3	52.57	7.65
Beauregrad	Organic (poultry pellet)	378.3b,b	4.3bc, a	18.36bc, ab	6.33abc, abc	34.8de,cd	79.3	27.59	7.21
	$NP_2O_5K_2O$ 100:100:100	328.1bc, bc	5.9a,a	17.47c,b	6.93ab,abc	42.6cd,bc	88.1	37.52	5.07
	$NP_2O_5K_2O$ 100:100:100 + 50 N	380.3b,b	4.7abc ,a	17.30c,b	5.71bcd, abc	38.9d,bcd	82.6	32.14	6.77
414	Organic (poultry pellet)	164.1d,d	5.1abc ,a	20.8ab,ab	3.7d,c	18.5f,e	54.1	9.32	9.21
	$NP_2O_5K_2O$ 100:100:100	273.9c,c	4.2bc, a	22.5a,a	4.31cd,bc	24.3ef,de	70.8	17.21	7.1
	$NP_2O_5K_2O$ 100:100:100 + 50 N	300.7c,bc	4.02c, a	21.90a,a	4.33cd,bc	25.2ef,de	67.3	16.97	8.25

*Different letters indicate a significant difference as determined by ANOVA, *factor significance < 0.05; factor significance ** <0.01.



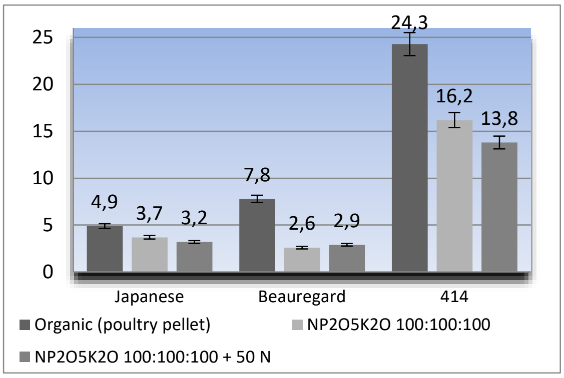


Chart.1. Percent (%) defects tuber (cracks, veins, constrictions or a predominance of pencil roots).

Chart 2. Percent (%) of small and tail roots

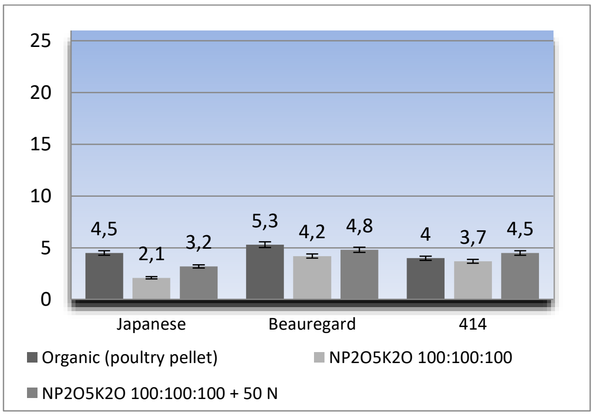


Chart 3. Percent (%) damage from soil pests

Chart 4. Sensory profiles created by the taste panel for sweet potato varieties

CONCLUSIONS

Sweet potatoes responded positively to increased doses of nitrogen fertilizers for all parameters of leaf mass yield in all cultivars in trials. However, the result of our research indicates that fertilization with organic poultry manure does not provide enough nutrients for optimal growth and development of tubers, while fertilization with complex mineral fertilizers with increased nitrogen content (150 N) represents a luxurious diet for sweet potatoes. The response to fertilization was significant in the case of tuber mass per plant, total tuber yield and leaf mass, while no effect was observed on the average tuber mass and tuber number. For optimal yield and quality of sweet potato tubers and leaves, we recommend fertilizing with $NP_2O_5K_2O$100:100:100. In our research, mineral nutrition ensures a uniform commercial yield in Japanese and Beauregard varieties, while it is significantly lower in 414 varieties. Based on the tasting panel, Beauregard received the highest rating. Japanese and 414 are less readily available for sale (mainly at local farms and markets), suggesting that advertising and tasting should attract consumers. Our analysis highlights the significant potential of sweet potato leaves and tubers, suggesting opportunities for expanded cultivation and increased consumer interest in this relatively new type of vegetable in Serbia.

FUNDING

This research was funded by the Ministry of Education Science and Technological Development of the Republic of Serbia with grant numbers 451-03-47/2023-01/200189.

REFERENCES

Aiuke F.O., Rao M.R., Swift M.J., Opondo-Mbai M.L. (2004). Effects oforganic and inorganic fertlizers on soil mineral nitrogen and maizeyields in western Kenya. In: sub-Saharan Africa. Bationo A (Ed.).Academy Science Publishers (ASP), AfNet-CIAT, Nairobi, Kenya pp.65-76.

Alam M.K., Rana Z.H., Islam S.N.(2016). Comparison of the proximatecomposition, total carotenoids and total polyphenol content of nineorange-fleshed sweet potato varieties grown in Bangladesh. Foods.5(3):1–10. doi: 10.3390/foods5030064.

Antonio, G.C., Takeiti, C.Y., Augustus De Oliveir, R., Park, K.J., 2011: Sweet potato: production, morphological and physicochemical characteristics, and technological process. Fruit Veg. Cereal Sci. Biotech. 5, 1-18.

Boeing H., Bechthold A., Bub A., Ellinger S., Haller D., Kroke A., Leschik-Bonnet E., Muller M.J., Oberritter H., Schulze M., Stehl P., Watzl, B. (2012) Critical review: Vegetables and fruit in the prevention of chronic diseases. Eur. J. Nutr.51:637–663. doi: 10.1007/s00394-012-0380-y.

Deksissa T., Short I., Allen J. (2008). Effect of soil amendment withcompost on growth and water use efficiency of Amaranth. In: Proc.the UCOWR/NIWR Annual Conference: International WaterResources: Challenges for the 21st century and water resourceseducation. July 22-24, 2008, Durham, North Carolina

Dinu M., Soare R., Becherescu A., Hoza Gh., Babeanu C. (2021). Bioactive compounds content and antioxidant activity in the leaves of some sweet potato cultivars (Ipomoea batatas L.). Scientific Papers. Series B, Horticulture. LXV, No. 1.

El Sheikha A.F., Ray R.C. (2017). Potential impacts of bioprocessing of sweet potato: review, Crit. Rev. Food Sci. Nutr. 57(3): 455–471.

Fernandes A.M. , Ribeiro N.P., Assunçao N.S. , Da Silva Nunes J.G. , Sorroche C.P. , Leonel M. (2021). Impact of nitrogen and green manure on yield and quality of sweet potato in sandy soil: A Brazilian case study..Journal of Agriculture and Food Research 4 100131.

Fernandes A.M., Campos L.G., Senna M.S., da Silva C.L., Assunçao N.S. (2018).Yield and Nitrogen Use Efficiency of Sweet Potato in Response to Cover Crop and Nitrogen Management. Agronomy Journal 110(5).

Filgueira F.A.R.(2008). Novo Manual de Olericultura: Agrotecnologia mod-erna na Produção e Comercialização de hortaliças New VegetableFarming Manual: Modern Agrotechnology in the Production andMarketing of Vegetables. 3rd edition. UFV

Gruneberg W.J., Espinosa J., Mwanga R.O.M., Andrade M., DapaahH. (2010). Procedures for the evaluations and analysis of sweetpotato trials.Maputo: CIP.https://www.researchgate.net/publication/325270781_Sweetpotato_production_processing_and_nutritional_quality

Huamán, Z. (1991). Descriptors for Sweet Potato. International Potato Center (CIP), Asian Vegetable Researchand Development Center (AVRDC) and International Board for Plant Genetic Resources (IBPGR),Lima

Khan M.U., Ahmed M., Shaukat S.S., Nazim K., Ali Q.M. (2013). Effect of industrial waste on early growth and phytoremediation potential of Avicennia marina. (Forsk.)Vierh.Pak.J.Bot.45(1):17-27

Kwak S.S.(2019).Biotechnology of the sweetpotato: ensuring global food and nutrition security in the face of climate change. Plant Cell Reports. 38:1361–1363

Leighton C., Schönfeldt H., Kruger R. (2010). Quantitative Descriptive Sensory Analysis of Five Different Cultivars of Sweet Potato to Determine Sensory and Textural Profiles

Liang N., Kitts D.D. (2016). Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions, Nutrients 8(1):16. https://doi.org/10.3390/nu8010016

Loebenstein, G., Fuentes, S., Cohen, J., Salazar, L.F. (2003) Sweet potato.In Viruses and Virus-Like Diseases of Major Crops in Developing Countries. (Loebenstein, G and G. Rhottapilly, eds.) Dortrecht:Kluwer Academic Publishers, pp. 223– 248

Mark B.P., John S.P., Craig A.A. (1983). Mobilization of nitrogen in fruitingplants of a cultivar of cowpea. J. Exp. Bot. 34(5):563-578.

Milenković L., Ilić S.Z., Stanojević Lj., Zvezdanović J., Sunić Lj. , Milenković A., Kevrešan Ž., Stanojević J., Cvetković D. (2024). Bioactive polyphenols of leaf biomass from sweet potato varieties under different fertilizers as a potential agri-food resource. Food of Science. 61-10457
Milenković L., Ilić Z.S., Šunić Lj., Lalević D. (2023). Uticaj sorte na morfološke osobine i prinos slatkog krompira (Ipomoea batatas L.) 125 godina primenjene nauke u poljoprivredi Srbije. Centar za strna žita i razvoj sela Kragujevac. Naučni skup nacionalnog karaktera. Zbornik radova. 172-182.
Nedunchezhiyan M., Byju G., Naskar S.K. Sweet potato (Ipomoeabatatas L).) as an intercrop in a coconut plantation: growth, yieldand quality. J Root Crops.33(1):26–9
Nsa S., Okon D., Robert S. (2013). Comparative effects of chicken manure and NPK on the yield of Ipomoea batatas. Journal of Agricultural and Crop Research, 1(6):90-93.
Padmaja, G. (2009). Uses and nutritional data sweetpotato. G. Loebenstein, G.Thottappilly (Eds.), The Sweetpotato, Springer Netherlends, Dordrecht (2009), pp.189-234.
Pedrosa, C.E., Andrade Júnior, V.C., Pinto, N.A.V.D., Figueiredo, J.A., Ribeiro, K.G., Azevedo, A.M., Lemos, V.T., Neiva, I.P., & Andrade, P.C.R. (2010). Seleção de clones de batata-doce com potencial de utilização na alimentação animal. Horticultura Brasileira , 28, 1974-1980.
RHMZ - Republički Hidrometeorološki zavod Srbije Kneza Višeslava 66 Beograd(hidmet.gov.rs)
Rice C.W. (2002). Organic matter and nutrient dynamics. In:Encyclopedia of soil science, Marcel Dekker, New York, USA. pp.925-928
Selemane N.R., Muetanene B.A., Jairoce C.F., Ricardo J., Francisco E.A., Ismael F. (2024). Evaluation of Some Sweetpotato(Ipomoea batatas [L.] Lam) Varietiesin Mozambique. European Journal of Agriculture and Food Sciences. 6 (4):22-26.
Suárez S., Mua T. , Suna H., Anón M.C. (2020).Antioxidant activity, nutritional, and phenolic composition of sweet potato leaves as affected by harvesting period. International Journal of Food Properties. 23(1):178–188 https://doi.org/10.1080/10942912.2020.1716796
Sun, H., Mu, T., Xi, L., Zhang, M., Chen, J. (2014). Sweet potato (Ipomoea batatas L.) leaves as nutritional and functional foods. Food Chemistry. 156: 380–389. http://dx.doi.org/10.1016/j.foodchem.2014.01.079 https://ifst.caas.cn/docs/2019-10/20191009115141356646.pdf
Taffouo V.D. (1994). Influence of mineral nutrient on growth, nutritionalvalue and yield of three tuber crops: comparative study. 3rd cyclethesis, University of Yaoundé 1:155.
Teow, C.C.; Truong, V.D.; McFeeters, R.F.; Thompson, R.L.; Pecota, K.V.; Yencho, G.C. (2007). Antioxidant activities, phenolic and β-carotene contents of sweet potato genotypes with varying flesh colours. Food Chem. 103, 829–838.
Todesco D., Moreira B.R.A., Barbosa Junior M.R., Maeda M., da Silva R.P. (2023). Sustainable management of sweet potatoes: A review on practices, strategies, and opportunities in nutrition-sensitive agriculture, energy security, and quality of life. Agricultural Systems. 210. 103693
Tomlins K.I., Rwiza E.J. , Ndengello T., Amour R. , Kapinga R.E., Rees D. (2003).The use of consumer tests and trained taste panels to assess sensory characteristics.Chapter 4, 13. https://www.researchgate.net/publication/240630896
Truong V.D., Avula R. Y., Pecota K. V., Yencho G. C.(2018). Sweetpotato Production, Processing, and Nutritional Quality. Handbook of Vegetables and Vegetable Processing. Botany and Physiology 2(2):811-838.

Udo D.J., Ndon B.A., Asuquo P.E., Ndaeyo N.U. (2005). Crop Production techniques for the tropics. Lagos, Nigeria, Concept production limited.
Villareal, R.L., Tsou, S.C., Lo, H.F., Chiu, S.C., 1982: Sweet potato tips as vegetables. In: Villareal, R.L., Griggs, T.D. (eds.), Sweet potato: Proceedings of the First International Symposium, 313–320. AVRDC: Shanhua; Taiwan.
Villordon, A. Q., La Bonte, D. R., Firon, N., Kfir, Y., Pressman, E., Schwartz, A. (2009). Characterisation of adventitious root development in sweetpotato. Horticultural Science 44: 651-655.
Wamba O.F., Taffouo V.D., Youmbi E., Ngwene B., Amougou A. (2012). Effects of organic and inorganic nutrient sources on the growth, total chlorophyll and yield of three bambara groundnut landraces in thecoastal region of Cameroon. J. Agron. 11(2):31-42.
Wolmann F.S., Guerra D., Souza E.L., Bohrer R.E.G., Bisognin R.P.,Lanzanova, Da Silva (2021). Productivity of three sweet potato cultivars:bRS Amélia, BRS Cuia and BRS Rubissol. Acta Agronómica;70:298–303.
Xu J.G., Hu Q.P., Liu Y. (2012). Antioxidant and DNA-protective activities of chlorogenic acidisomers,J.Agric.Food.Chem.60(46):11625–11630. https://doi.org/10.1021/jf303771s
Yu Y., Wang Y., Yu Y., Ma P., Jia Zh., Guo X., Xie Y., Bian X.(2020). Over expression of IbPAL1 promotes chlorogenic acid biosynthesis in sweetpotato Crop Journal 9(1).

CONTROL OF THE HOUSE MOSQUITO *CULEX PIPIENS* USING A PLANT EXTRACT

Gacem Habiba[1,2], Khafallah Imene[1,3], Rizi Hadia[4], Amira Khedidja[1,3], Djeghader Nour El-Houda[3,5]*

[1] *Higher Normal School of Technological Education, skikda, 21000, Algeria.*
[2]*Laboratory of Biology, Water and Environment, University of 08 May 1945,Guelma, 24000, Algeria*
[3] *Laboratory of physical, Chemistry and Biology of Materials, Skikda, 21000, Algeria*
[4] *Department of Biology, Faculty of Sciences and Nature, Chadli Bendjedid University of El-Tarf, 36000, Algeria.*
[5]*Department of Biology, Faculty of Sciences, University of BADJI Mokhtar, Annaba, 23000, Algeria.*

Corresponding author e-mail: ritadjbiba7@gmail.com

ABSTRACT
Mosquitoes are the first cause of transmission of viral diseases to both humans and animals, as they can transmit some viral diseases. These insects have attracted the attention of specialists, especially in the field of control, because they have controlled their spread in urban areas. Traditionally, they have resorted only to insecticides to eliminate them, and aquatic organisms have long been used to assess the health of aquatic systems. Biodiversity refers to the diversity of organisms in a given area or ecosystem, including species diversity, genetic diversity, and ecosystem diversity including terrestrial, marine, and other aquatic ecosystems. It is important for maintaining healthy and stable ecosystems, as well as for providing humans with food, medicine, and other resources. Mosquito larvae are the immature form of mosquitoes that live in water. It feeds on microorganisms and organic matter in the water and undergoes several molts as it grows and develops into a pupa and then an adult mosquito. The aim of our work is to investigate the effect of 'rosemary oil' on a target species, the larvae of the *Culex pipiens* mosquito. And to determine its effectiveness as an alternative insecticide in order to control mosquito larvae, while reducing the effect on non-target species. In the light of these findings, rosemary oil is considered an effective means to control mosquito larvae and has positive effects on non-target organisms. Its use as a natural pesticide can promote sustainable pest control practices. So it is considered a biological insecticide.
Keywords: Insecticide, Larvae, Extract, Plant, *Culex pipiens*, Mosquito

INTRODUCTION

The diversity of organisms in a particular area or ecosystem, including species diversity, genetic diversity, and ecosystem diversity including terrestrial, marine, and other aquatic ecosystems. It is important for maintaining the health and stability of ecosystems, as well as providing humans with food, medicine and other resources. The goal of our work is to combat the domestic mosquito *Culex pipiens* using the plant extract “rosemary oil”. This study aims to determine the effectiveness of “rosemary oil” as an alternative to insecticides in combating mosquito larvae while reducing its impact on non-target species **(Kramer et al., 2019).**

MATERIAL AND METHOD

Presentation of the study area Garaate Djamel

Garaate Djamel is located in the commune of El Chat, daira of Ben Mhidi on the left side of the wilaya road number 109 connect the wilaya of Annaba with El- Kala, and it's located at 36° .88 N and 07° .90 E. This body of water does not discharge into the sea water which minimizes their variation under the effect of currents (Figure 1).

Figure 1. Location of observation station (Gacem, 2023)

Identification of The *Culex pipiens pipiens* (Northern house mosquito)

Systematic position of the *Culex pipiens pipiens*:

Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Diptera
Family: Culicidae
Genus: Culex
Subgenus: Culex
Species: *Culex pipiens*
Subspecies: *Culex pipiens pipiens*

Figure 2. *Culex pipiens pipiens* (personal photo)

Identification of The Rosemary: Rosemary essential oil is a clear to pale yellow liquid that is extracted through steam distillation, typically using direct steam, from the flowers, leaves, and twigs of the Rosemary plant

Systematic position of The Rosmary

Kingdom: Plantae
Subkingdom: Tracheophytes-Vascular plants
Superdivision: Spermatophyta-seed plants
Division : Magnoliophyta-flowering plants
Class: Magnoliopsida-Dicotyledons
Subclass: Asteridae
Order: Lamiales
Family: Lamiaceae
Genus: Salvia
Species*:* *S. rosmarinus*

The Rosmarry oil tests

- The Daphnia and The *Culex pipiens pipiens* larvae were collected from Garaat Djamel lake.
- From the prepared oil (Rosmarry oil). The tests are carried out in plastic boxes (Used for butter conservation) of 500 ml volume . 2 tests of 2 different insects are carried out to determine the effect of the Rossmarry oil. Each test is carried out in 3 repetition and a control.
- The first test concerned 10 individuals of the *Daphnia magna*, 200 ml of the lake water and 0.2 ml of " ROSEMARY OIL" .
- The second test concerned 20 individuals of The *Culex pipiens* Mosquito larva, 200 ml of the lake water and 0.2 ml of " ROSEMARY OIL".
- Mortality was assessed after 24h, 48h and 72h.

Statistical analysis

The results are presented by the mean ± standard deviation and by the percentage of mortality. We used Exel 2010.

RESULTS AND DISCUSSION

For results based on standard deviation and mean:

We conducted a statistical analysis using Excel 2010, which showed that there is a difference between the effect of “rosemary oil” on the species, and the values are summarized in Table 04 and represented in Figure 39. After 24 hours of treatment with “rosemary oil”, the results showed that the average mortality rate in Daphnia larvae was lower than the average mortality rate in Daphnia larvae.

Table 1: The results of the effect of the ROEMARY OIL during 72h ; M±S.

Time / species	24h	48h	72h
Daphnia	1.66±0.57	0±0	0±0
C. Larvae	8.33±1.52	0±0	0±0

A study of "Antimicrobial activity of Rosmarinus officinalis L. essential oil against Gram-positive and Gram-negative bacteria and Candida spp" found that "ROSEMARY OIL" had significant antimicrobial activity against a variety of target species, including Gram-positive and Gram-negative bacteria and Candida spp. The researchers suggested that rosemary oil may be a promising alternative to conventional antibiotics and antifungal agents.(Silva et al.,2018)

A study on “Antimicrobial activity of Rosmarinus officinalis L. essential oil against Gram-positive and Gram-negative bacteria and Candida spp” found that “Rosemary oil” has

Significant antimicrobial activity against a variety of target species, including Gram-positive and Gram-positive. Negative bacteria and Candida spp. The researchers suggested that rosemary oil may be a promising alternative to traditional antibiotics and antifungal agents.

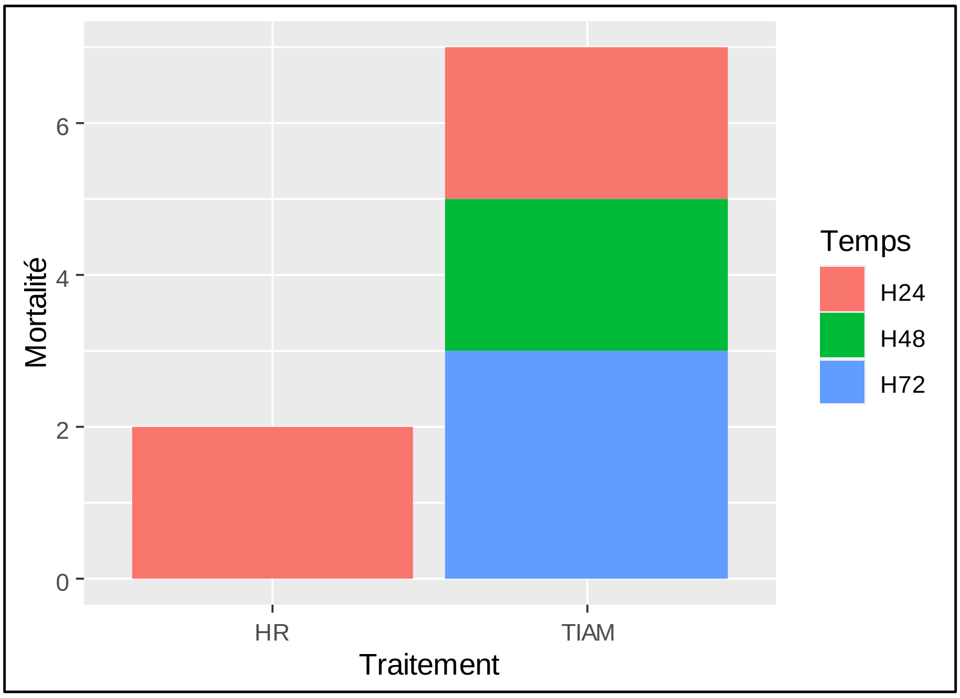


Figure 4: The effect of the "TIAM" insecticide and the "ROSEMARY OIL".

CONCLUSIONS

A study on "Antimicrobial activity of Rosmarinus officinalis L. essential oil against Gram-positive and Gram-negative bacteria and Candida spp" found that "Rosemary oil" has significant antimicrobial activity against a variety of target species, including Gram-negative bacteria and Candida spp. The researchers suggested that rosemary oil may be a promising alternative to traditional antibiotics and antifungal agents.

REFERENCES

Gacem, H., Bendali-saoudi, F., Serradj, N., Houmani, M., & Soltani, N. (2023). Risk assessment of the neonicotinoid insecticide acetamiprid on two non-target species, daphnia magna straus, 1820 (crustacea, cladocera) and plea minutissima leach, 1817 (insecta, heteroptera). Applied Ecology & Environmental Research, 21(2).

Silva, N. C., et al. 2018. Brazilian Journal of Microbiology, 49(4), 968-973.

Gacem H, 2022, Imen K, Rabeh C, Lina L. 2022. A comparative study between biological and chemical control against domesticmosquito larvae. Biodiversitas 23: 6456-6462

EFFECTS OF ACTIVE SUBSTANCE CHLOROTOLURON ON WHEAT

Deniz AŞKAR[1], Hayat TOPÇU[2], Behiye Banu BİLGEN[2]

[1] Tekirdağ Namık Kemal University, Graduate School of Natural and Applied Sciences, Agricultural Biotechnology Department, Tekirdağ, Turkey
[2] Tekirdağ Namık Kemal University, Faculty of Agriculture, Agricultural Biotechnology Department, Tekirdağ, Turkey
Corresponding author e-mail: bbilgen@nku.edu.tr

ABSTRACT

Wheat (*Triticum aestivum* L.) plays a critical role in global agriculture, and herbicides used for weed control, particularly chlorotoluron, have significant effects on wheat. Although chlorotoluron is commonly used to control weeds in wheat, excessive use can lead to accumulation in the soil and toxicity in plants. Studies have shown that chlorotoluron creates oxidative stress in wheat plants, disrupting photosynthesis and negatively affecting plant growth. Additionally, organic matter in the soil that influences the absorption of chlorotoluron can reduce its accumulation and toxicity in plant tissues. However, further research on the toxic effects of chlorotoluron in plants and its environmental behavior is needed to understand the effects of herbicides better and reduce environmental damage. For these reasons, this review aims to provide information on studies and general knowledge related to chlorotoluron in wheat conducted to date.

Key words: Chlorotoluron, Herbicides, *Triticum aestivum* L.

INTRODUCTION

Wheat (*Triticum aestivum* L.) has the highest grain production among all cultivated food crops, with 808.4 Mt produced in 2022, and it covers approximately 219.1 Mha of arable land, making a significant contribution to the global agricultural economy (FAO, 2022). As arable land decreases, sustainable wheat production becomes crucial for global food security. In the development processes of wheat, weed infestation and control represent significant obstacles and threats to wheat production worldwide. The use of herbicides for weed management in both uncultivated soil and fallow areas is highly preferred in developed countries. As a result of the widespread and intensive use of herbicides, the evolution and spread of herbicide-resistant weed species have been observed in many crop production systems, including wheat. According to the International Herbicide-Resistant Weeds Survey, wheat ranks first worldwide in reported resistance cases, with a total of 77 weed species exhibiting resistance to 140 different herbicides (Nakka et al. 2019).

The use of herbicides in agricultural fields is one of the most important practices for controlling weed growth in modern farming. However, recent intensive use of herbicides has led to significant accumulation of these substances in the soil. As herbicides have become one of the most commonly observed organic pollutants in agricultural areas, there are growing concerns about their potential impacts on produced crops, ecosystems, and human health (Song et al. 2007).

The fundamental challenge that agriculture currently faces and will continue to face in the future is providing food for the world's growing population while protecting the environment. Increases in crop yield are often based on the use of pesticides. The competition between plants and weeds makes herbicides the most commonly used type of pesticide for soil nutrients, thereby promoting plant growth and enhancing yield (Marín-Benito et al. 2019).

Herbicides possess physical and chemical properties that allow for their absorption upon initial contact with plant tissues. They are then transported to target areas, where they can cause injury and death in susceptible weed species or cultivated plants. The control of a weed species depends on the mode of action of the herbicide. The mode of action is defined as a series of biochemical and physiological processes that begin when the plant comes into contact with the herbicide and continue until the plant's complete death (Nakka et al. 2019).

Chlorotoluron is a phenylurea herbicide widely used for controlling weeds in cereal, fruit, and cotton production. However, excessive use of this herbicide can lead to its accumulation in ecosystems, thereby causing toxicity in plants. Chlorotoluron [3-(3-chloro-4-methylphenyl)-1,1-dimethylurea], a phenylurea herbicide with high activity and relatively low toxicity, is commonly used to control weeds during production. It is typically applied to the soil and is moderately soluble in water, which allows it to accumulate easily in plants (Song et al. 2007). This substance is particularly effective against broadleaf weeds. Chlorotoluron is a herbicide with low mobility in soil and can therefore be effective for a long time after application to the soil (Smith, J. and Brown, T. 2021).

Effects of Chlorotoluron Active Ingredient on Wheat

Wheat is one of the most widely cultivated crops globally. When weeds are not controlled in wheat fields, yield losses can range from 10% to 50%, depending on the density of the weeds and the duration of the infestation. Herbicide applications for weed control have proven to be very effective and efficient in terms of production costs and benefits. However, repeated use of herbicides for weed management has led to selection pressure and, consequently, the evolution of herbicide-resistant weed species (Nakka et al. 2019).

In wheat, natural resistance to different herbicides varies depending on factors such as variety, application timing (pre-emergence, pre-planting, or post-emergence), soil and environmental conditions, and the use of protective compounds. Therefore, wheat growers should carefully follow label recommendations to prevent crop damage. Typical herbicide damage symptoms in wheat can range from yellowing of leaf tips to reduced spike development and may show more severe symptoms depending on the mode of action of the herbicide (Nakka et al. 2019).

Chlorotoluron provides effective weed control, especially during the wheat growing season. This allows the wheat plant to use its nutrient and water resources more efficiently. However, it should be noted that chlorotoluron may not be effective against some weed species and may need to be combined with other control methods (Smith, J. and Brown, T. 2021).

A study conducted in 1993 examined the metabolism of the herbicide chlorotoluron in wheat cell suspension cultures. The study used the sensitive variety Corin (S) and the tolerant variety Clément (T), along with six genetically similar isogenic lines (9S, 10S, 16S, 17T, 18T, and 24T). These isogenic lines have the genetic background of the Chinese Spring variety, with the 17T, 18T, and 24T lines containing a Sul tolerance gene transferred from the Cappelle-Desprez variety. Both Clément and Corin cell cultures produced similar metabolite patterns. The metabolism rate of chlorotoluron is significantly higher in Clément cell cultures compared to Corin cell cultures. Among the isogenic lines, metabolite patterns are similar, with metabolism rates being equal in 9S, 10S, 16S, 18T, and 24T, but lower in 17T. These findings suggest that there is no different acceleration mechanism between T and S cell cultures and that the selectivity of wheat varieties towards chlorotoluron is related to differences in metabolism (Cabanne ve Snape, 1993).

In a study conducted by Krugman et al. (1997) on bread wheat and wild wheat plants, RFLP markers were used to map the chlorotoluron resistance gene (Su1) in sensitive and resistant plants. The results of the study indicate that the Su1 gene, which responds differently to chlorotoluron, evolved before the wheat was cultivated and did not develop as a reaction to the chemical use.

In a study conducted by Song et al. (2007), a series of experiments were performed to evaluate the toxicity of chlorotoluron in wheat plants (*Triticum aestivum* L.) and to assess the metabolic adaptation to chlorotoluron-induced oxidative stress. Wheat plants were grown in soils containing chlorotoluron at concentrations of 0-25 mg/kg. Chlorotoluron accumulation in the plants showed a positive correlation with the external chlorotoluron concentrations but a negative correlation with plant growth. Chlorotoluron application caused the accumulation of O2− and H2O2 in the leaves and led to plasma membrane lipid peroxidation in the plants. Endogenous proline levels were measured and found to accumulate significantly in the roots and leaves of plants exposed to chlorotoluron.

In a study conducted in 2008, the effects of sludge (SL) and straw (ST) extracts on the absorption and mobility of chlorotoluron in soils were investigated. Applications of SL and ST dissolved organic matter (DOM) reduced the absorption of chlorotoluron and increased its desorption. The addition of DOM increased the mobility of chlorotoluron and decreased its accumulation in plant tissues. FT-IR and fluorescence analyses indicated that ST DOM contained more unsaturated components and amines. These results may help in understanding the role of DOMs in the environmental behavior of organic chemicals (Song et al. 2008).

CONCLUSION AND RECOMMENDATIONS

Ecological factors that are harmful to plants, such as heavy metals, herbicides, pesticides and organic pollutants, can lead to physiological and oxidative stress. Currently, the mechanisms of herbicide-induced oxidative stress have not yet been fully elucidated. To utilize of chlorotoluron importantly push down the emergence of chlorophyll, break the photosynthesis of aim plants (Song et al. 2007). Morever, studies have stated that organic matter applications can reduce the rate and strength of chlorotoluron in plants (Song et al. 2010).

To date, very few studies have been conducted on chlorotoluron. Current studies are not sufficient to completely figure out the mechanisms of oxidative stress reasoned by herbicides, nor are they adequate to fully grasp chlorotoluron. More studies should be performed to learn the treatment of this phenylurea herbicide in plants, its gathering in the plant and soil, the damages of its toxicity, and whether it causes genetic damage in subjected plants.

REFERENCES

FAO, 2024. Food and Agricultural Organization. www.FAO.org.

Marín-Benito, J. M., Carpio, M. J., Sánchez-Martín, M. J., & Rodríguez-Cruz, M. S. (2019). Previous degradation study of two herbicides to simulate their fate in a sandy loam soil: Effect of the temperature and the organic amendments. *Science of The Total Environment*, *653*, 1301-1310. https://doi.org/10.1016/J.SCITOTENV.2018.11.015

Nakka, S., Jugulam, M., Peterson, D., & Asif, M. (2019). Herbicide resistance: Development of wheat production systems and current status of resistant weeds in wheat cropping systems. *The Crop Journal*, *7*(6), 750-760. https://doi.org/10.1016/J.CJ.2019.09.004

Song, N. H., Chen, L., & Yang, H. (2008). Effect of dissolved organic matter on mobility and activation of chlorotoluron in soil and wheat. *Geoderma*, *146*(1-2), 344-352. https://doi.org/10.1016/J.GEODERMA.2008.05.031

Song, N. H., Yin, X. Le, Chen, G. F., & Yang, H. (2007). Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. *Chemosphere*, *68*(9), 1779-1787. https://doi.org/10.1016/J.CHEMOSPHERE.2007.03.023

Song, N. H., Zhang, S., Hong, M., & Yang, H. (2010). Impact of dissolved organic matter on bioavailability of chlorotoluron to wheat. *Environmental Pollution*, *158*(3), 906-912. https://doi.org/10.1016/J.ENVPOL.2009.09.019

Smith, J., and Brown, T. (2021). *Herbicide effects on crop growth and yield. Journal of Agricultural Science, 45(3), 123-134.*

AGRICULTURAL LAND USE SITUATION AND CHEMICAL FERTILIZER CONSUMPTION IN VAN PROVINCE

*Bulut SARĞIN, Siyami KARACA, Füsun GÜLSER**

Van Yuzuncu Yıl University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Van, Türkiye
Corresponding author e-mail: fgulser@yyu.edu.tr

ABSTRACT

Significant changes were observed in agricultural land use, harvested products and fertilizer consumption between 2014 and 2023 in Van province. While the table grape production area was 320 decares in 2014, it increased to 633 decares in 2023 by an increase of 98 %. Table grape production increased from 78 tons in 2014 to 388 tons in 2023, an increase of 397%. While the apple cultivation area was 24,176 decares in 2014, it increased to 27,785 decares in 2023. While apple production was 7379 tons in 2014, it reached 17632 tons in 2024.While pear production was 1968 tons in 2014, it increased by 57% to 3102 tons in 2023. Watermelon production area, which was 1730 decares in 2014, increased to 1904 decares in 2023. While watermelon production was 4322 tons in 2014, it increased to 6317 tons in 2023 and showed a general increasing trend. While the melon production area was 1842 decares in 2014, it reached 2450 decares in 2023. Melon production increased similarly, reaching 6901 tons in 2023, while it was 3203 tons in 2014.A large increase was observed in the tomato production area which was 4811 decares in 2014 and increased to 9706 decares in 2023. In the same time period, tomato production increased from 11,212 tons to 53039 tons. While the wheat production area was 811674 decares in 2014, it decreased to 645758 decares in 2023, and the barley production area increased from 70824 decares to 451834 decares in the same period. Barley production increased from 94779 tons in 2014 to 109203 tons in 2023. Wheat production increased in a similar way, reaching 61906 tons in 2023, while it was 11364 tons in 2014. While the potato production area was 1666 decares in 2014, it increased to 1692 decares in 2023. While potato production was 4006 tons in 2014, it increased to 5875 tons in 2023, and it generally shows an increasing trend. The sugar beet production area, which was 6610 decares in 2014, increased and reached to 24,666 decares in 2023. Sugar beet production, which was 35755 tons in 2014, increased to 114475 tons in 2023.The sainfoin production area, which was 200,713 decares in 2014, increased to 309,800 decares in 2023. Sainfoin production increased from 107,777 tons in 2014 to 363,152 tons in 2023. A decrease was determined in chickpea, onion and alfalfa production areas between 2014 and 2023. Although the walnut production area increased in the same time period, a decrease in the amount of product was determined. Considering chemical fertilizer consumption, the consumption of urea, ammonium sulfate, calcium ammonium nitrate and ammonium nitrate fertilizers was 879 tons, 149 tons, 411 tons and 1243 tons, respectively, in 2014.In 2023, the consumption of these fertilizers increased and the consumption of urea, ammonium sulfate, calcium ammonium nitrate fertilizers was 1758 tons, 262 tons and 11050 tons, respectively. The consumption of diammonium phosphate fertilizer decreased from 1400 tons in 2014 to 728 tons in 2023.

Keywords: Land use, agricultural production, chemical fertilizer,Van province

INTRODUCTION

Over the last few decades, global agricultural production has increased, and advances in technology are still contributing to increased yields. However, population growth, climate change, water crisis and deforestation threaten global food security (Najafi et.al., 2018).

Soil fertility is the ability of the soil to support crop production, determined by the interaction of its physical, chemical and biological properties and usually measured in terms of yield (Lontoc-Roy et al., 2006).

The main purpose of applying fertilizer to the soil is to improve the nutritional status and quality of the soil by enriching it with missing nutrients (Mani 2002). Fertilizers increase soil fertility so that crop yields are no longer limited by missing amounts of plant nutrients (Cooke,1982). Since the nutrients in mineral fertilizers are relatively high and the release of these nutrients is rapid, there is no need for direct decomposition. Therefore, inorganic fertilizers more rapidly increase the growth rate and plant's overall productivity (Ojeniyi, 2002).

In this study investigated that agricultural land use, crop yield and fertilizer consumption between 2014 and 2023 in Van province.

LAND USE AND CROP YIELDS AMONG 2014 -2023 YEARS IN VAN PROVINCE

Significant changes were observed in agricultural land use, crop yield and fertilizer consumption between 2014 and 2023 in Van province. While the table grape production area was 320 decares in 2014, it increased to 633 decares in 2023 by an increase of 98 %. Grape production increased from 78 tons in 2014 to 388 tons in 2023, an increase of 397% (Figure 1).

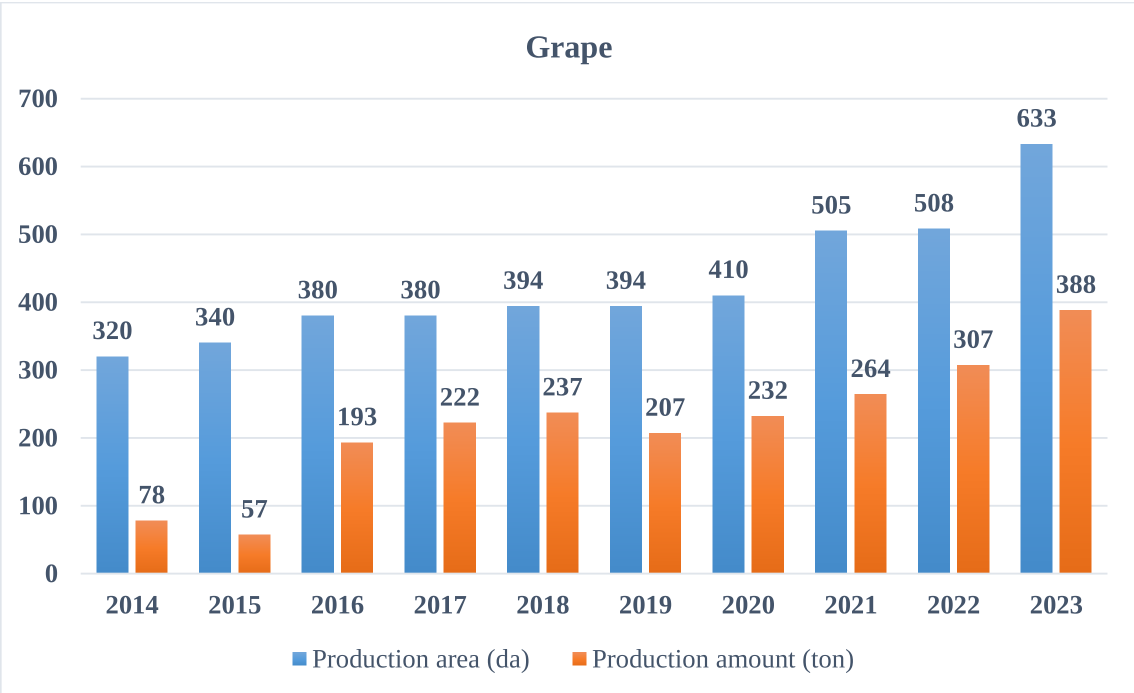


Figure 1. Grape production area and production amount (TUİK 2023)

While the apple cultivation area was 24.176 decares in 2014, it increased to 27.785 decares in 2023. While apple production was 7379 tons in 2014, it reached 17632 tons in 2024 Figure 2).

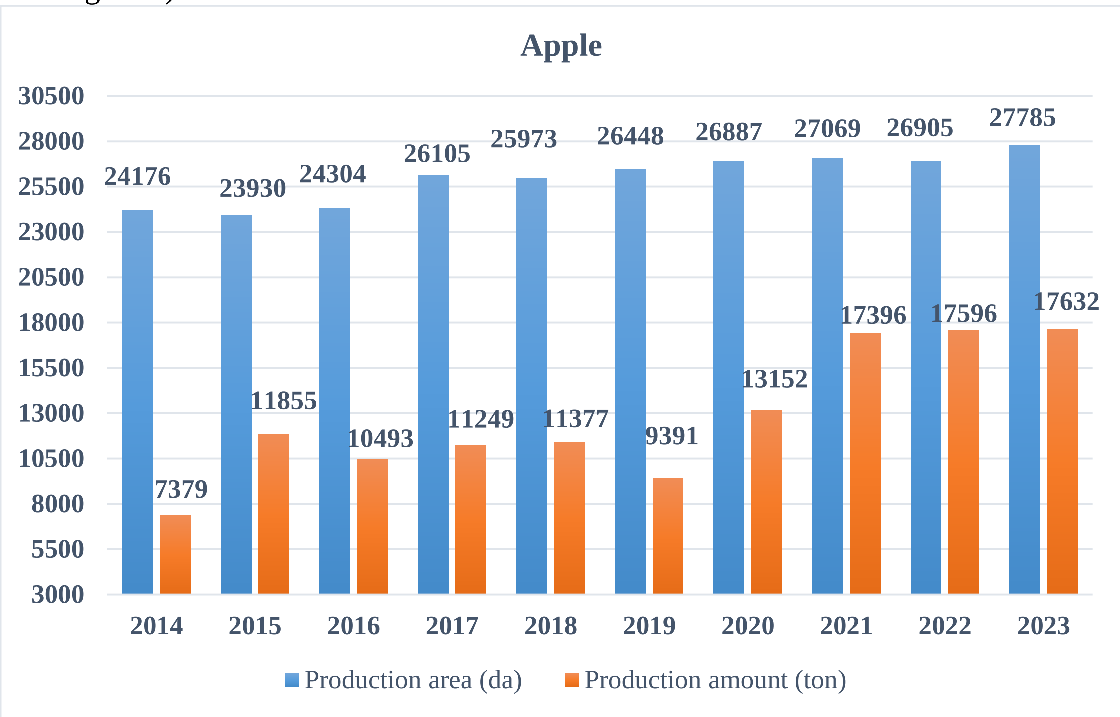


Figure 2. Apple production area and production amount (TUİK 2023)

While pear production was 1968 tons in 2014, it increased by 57% to 3102 tons in 2023 (Figure 3).

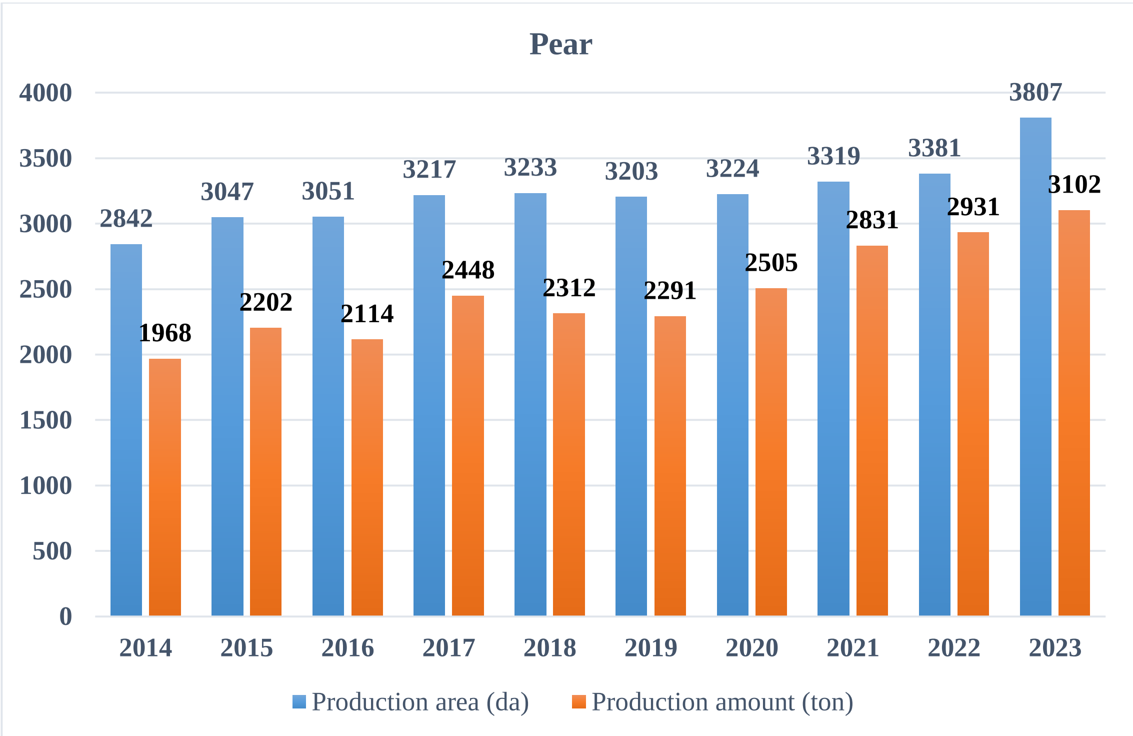


Figure 3. Pear production area and production amount (TUİK 2023)

Although the walnut production area was 2842 decares in 2014 and reached 3807 decares in 2023, walnut production, which was 8001 tons in 2014, decreased slightly to 6817 tons in 2023 (Figure 4).

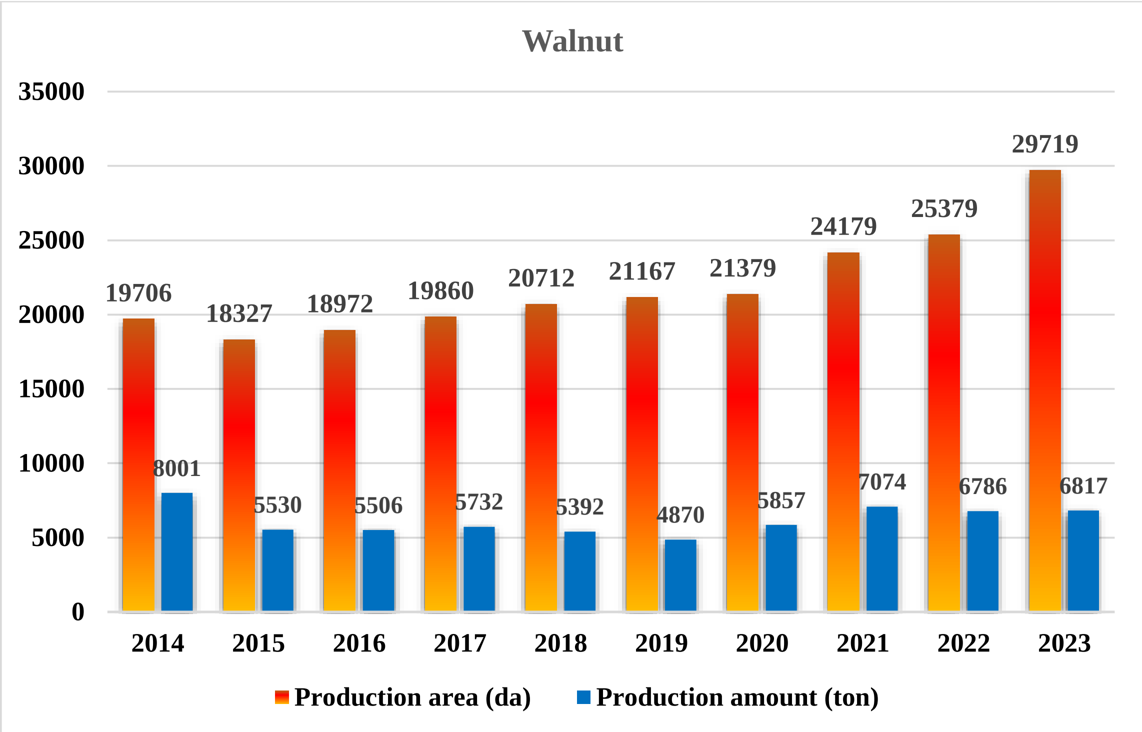


Figure 4. Walnut production area and production amount (TUİK 2023)

Watermelon production area, which was 1730 decares in 2014, increased to 1904 decares in 2023. While watermelon production was 4322 tons in 2014, it increased to 6317 tons in 2023 and showed a general increasing trend (Figure 5).

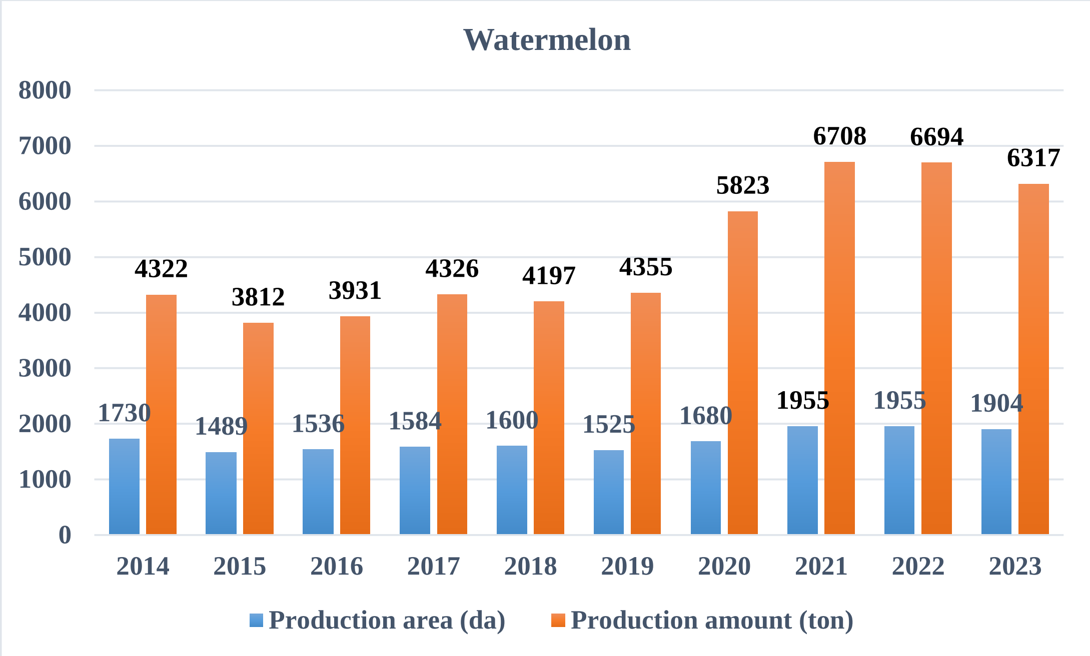


Figure 5. Watermelon production area and production amount (TUİK 2023)

While the melon production area was 1842 decares in 2014, it reached 2450 decares in 2023. Melon production increased similarly, reaching 6901 tons in 2023, while it was 3203 tons in 2014 (Figure 6).

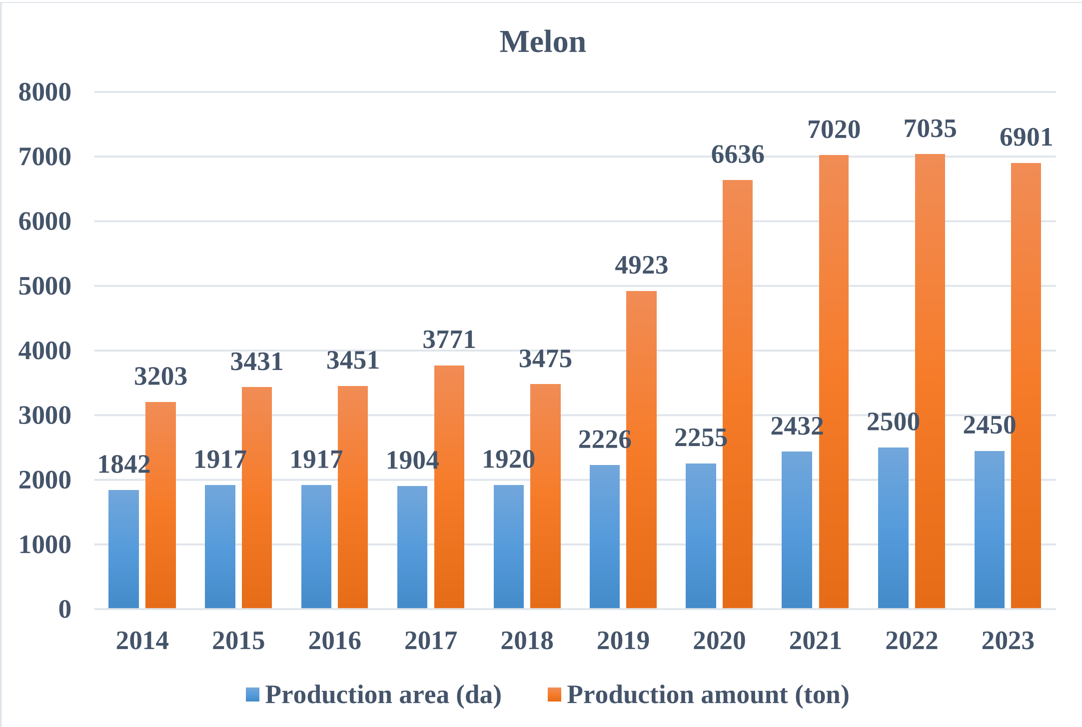


Figure 6. Melon production area and production amount (TUİK 2023)

A large increase was observed in the tomato production area which was 4811 decares in 2014 and increased to 9706 decares in 2023. In the same time period, tomato production increased from 11,212 tons to 53039 tons (Figure 7). This shows that the agricultural and economic importance of tomatoes is increasing (Kumbasaroğlu et all, 2021).

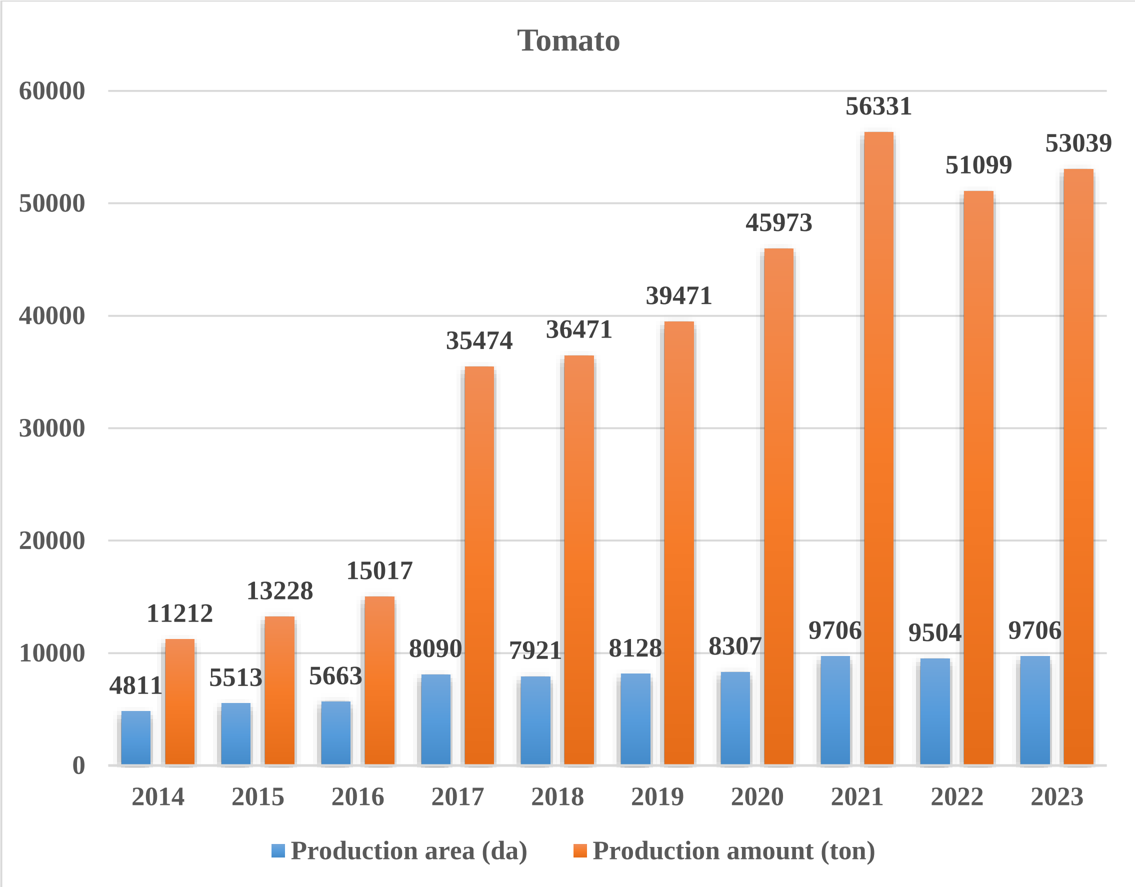


Figure 7. Tomato production area and production amount (TUİK 2023)

While the wheat production area was 811674 decares in 2014, it decreased to 645758 decares in 2023, and the barley production area increased from 70824 decares to 451834 decares in the same period (Figure 8).

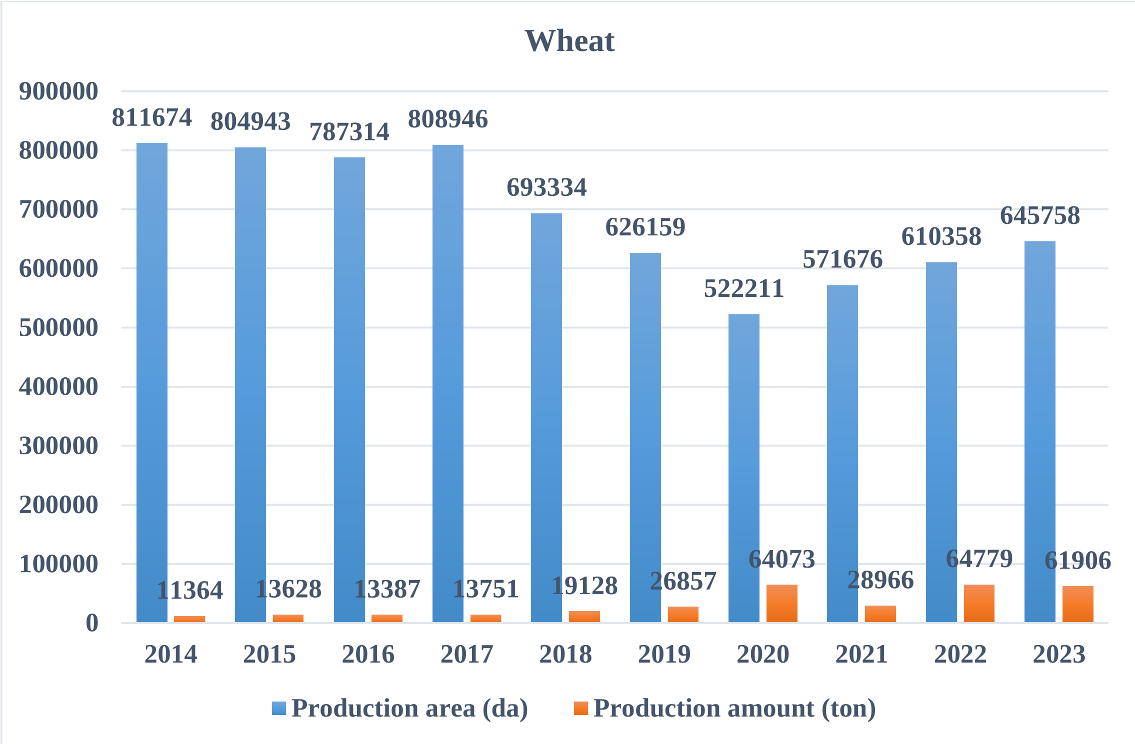


Figure 8. Wheat production area and production amount (TUİK 2023)

Barley production increased from 94779 tons in 2014 to 109203 tons in 2023. Wheat production increased in a similar way, reaching 61906 tons in 2023, while it was 11364 tons in 2014 (Figure 9). This large increase in barley production, in particular, shows that the agricultural value and demand of barley has increased (Kaya and Demir, 2021).

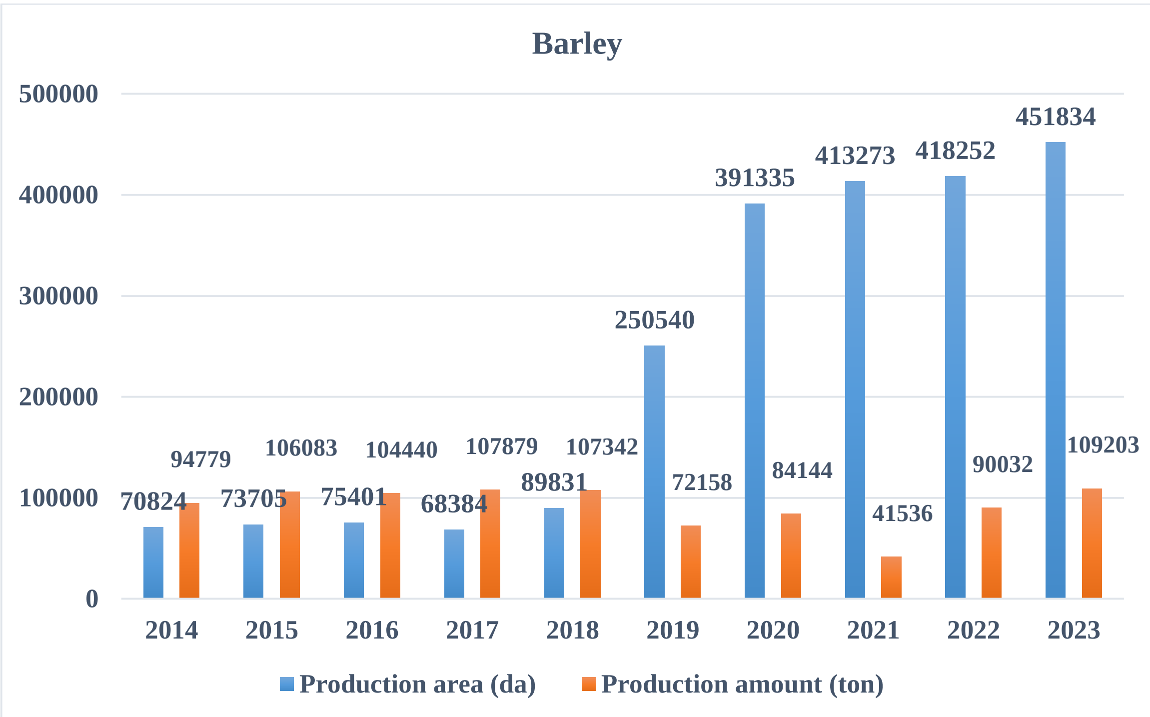


Figure 9. Barley production area and production amount (TUİK 2023)

Chickpea production area followed a fluctuating course, starting with 1620 decares in 2016 and decreasing to 1009 decares in 2023 (Figure 10). While chickpea production was 195 tons in 2016, it decreased to 130 tons in 2023.

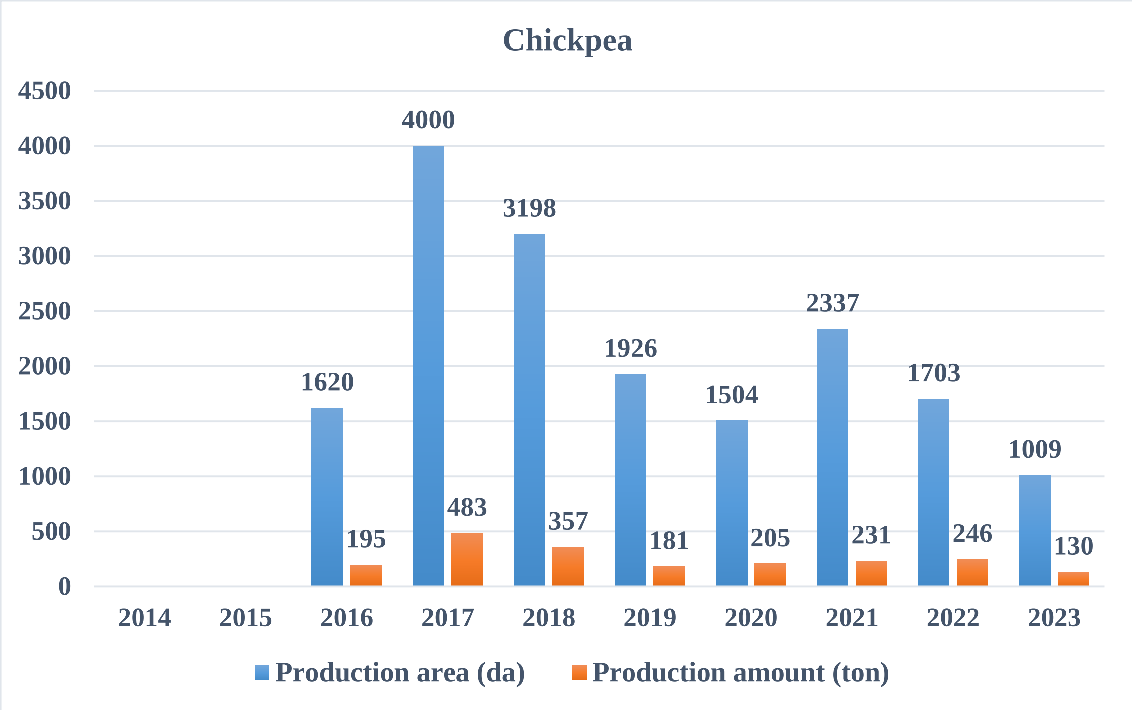


Figure 10. Chickpea production area and production amount (TUİK 2023)

While the potato production area was 1666 decares in 2014, it increased to 1692 decares in 2023. While potato production was 4006 tons in 2014, it increased to 5875 tons in 2023, and it generally shows an increasing trend (Figure 11).

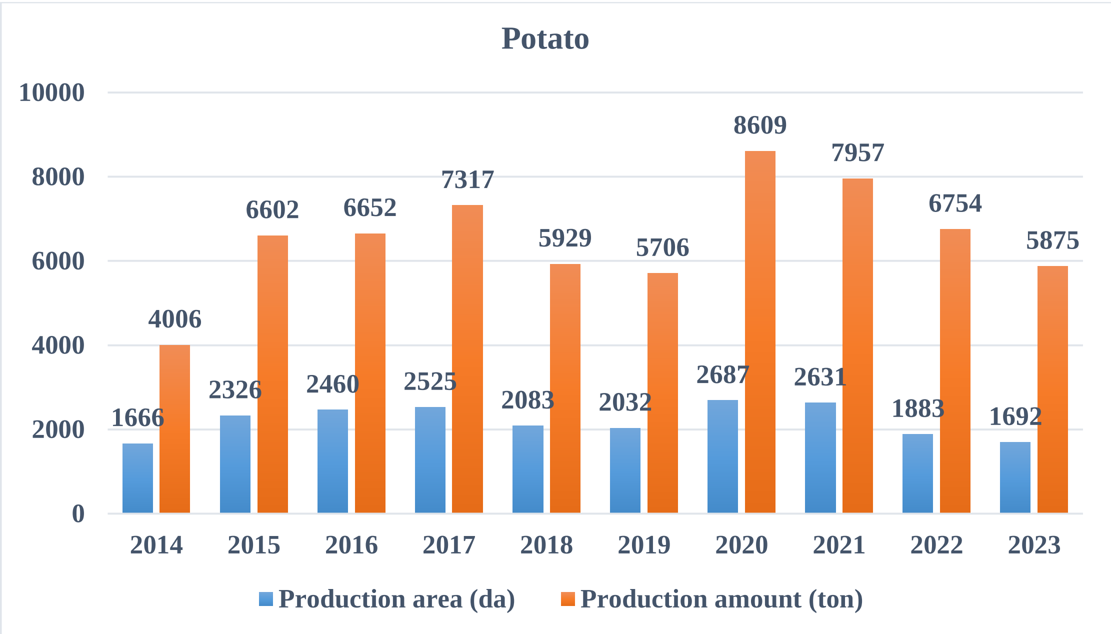


Figure 11. Potato production area and production amount (TUİK 2023)

However, the onion production area decreased from 773 decares in 2014 to 408 decares in 2023. While onion production was 641 tons in 2014, it decreased to 546 tons in 2023. The highest onion production was determined as 1082 tons in 2016 (Figure 12).

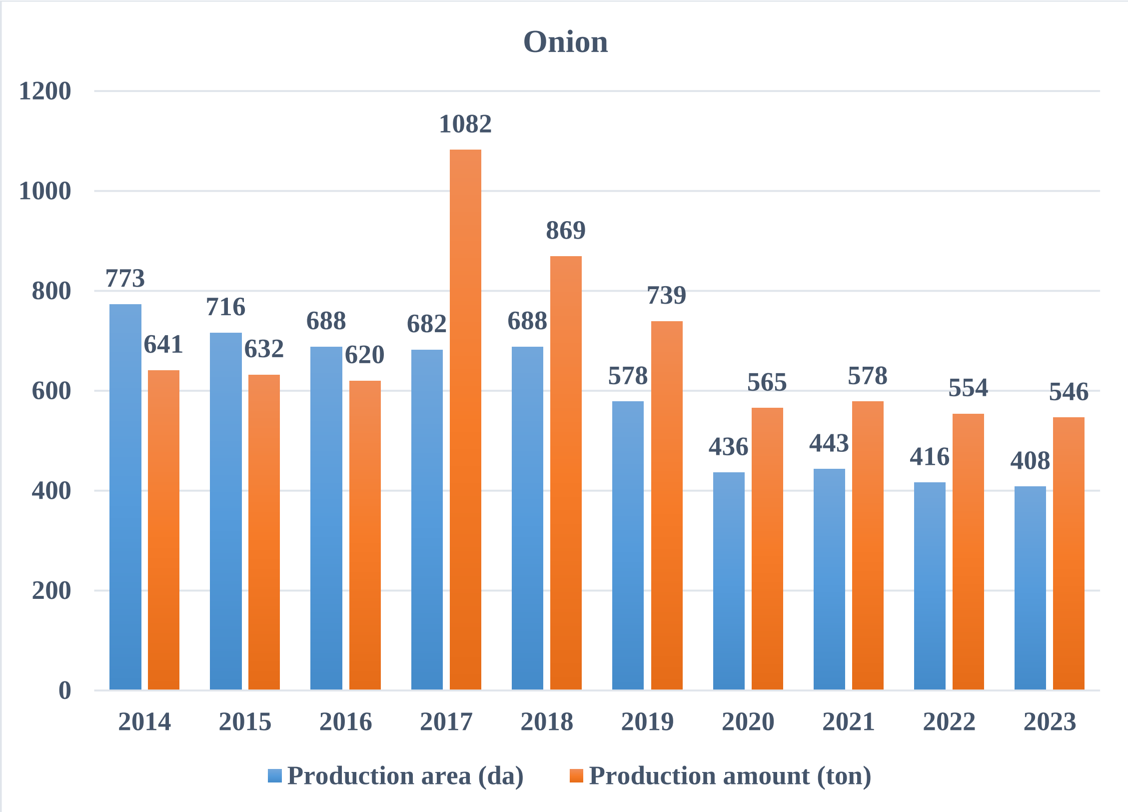


Figure 12. Onion production area and production amount (TUİK 2023)

The sugar beet production area, which was 6610 decares in 2014, increased and reached to 24666 decares in 2023. Sugar beet production, which was 35755 tons in 2014, increased to 114475 tons in 2023.The sainfoin production area, which was 200713 decares in 2014, increased to 309800 decares in 2023 (Figure 13).

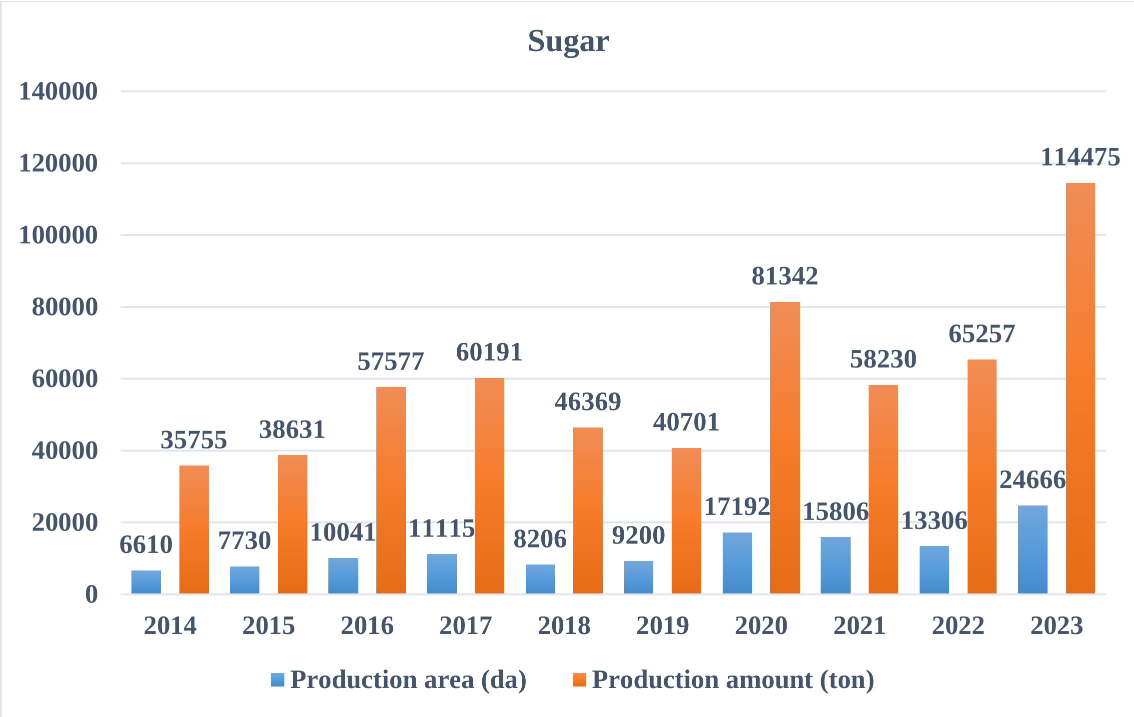


Figure 13. Sugar production area and production amount (TUİK 2023)

The sainfoin production area, which was 200713 decares in 2014, increased to 309800 decares in 2023. Sainfoin production increased from 107777 tons in 2014 to 363152 tons in 2023 (Figure 14).

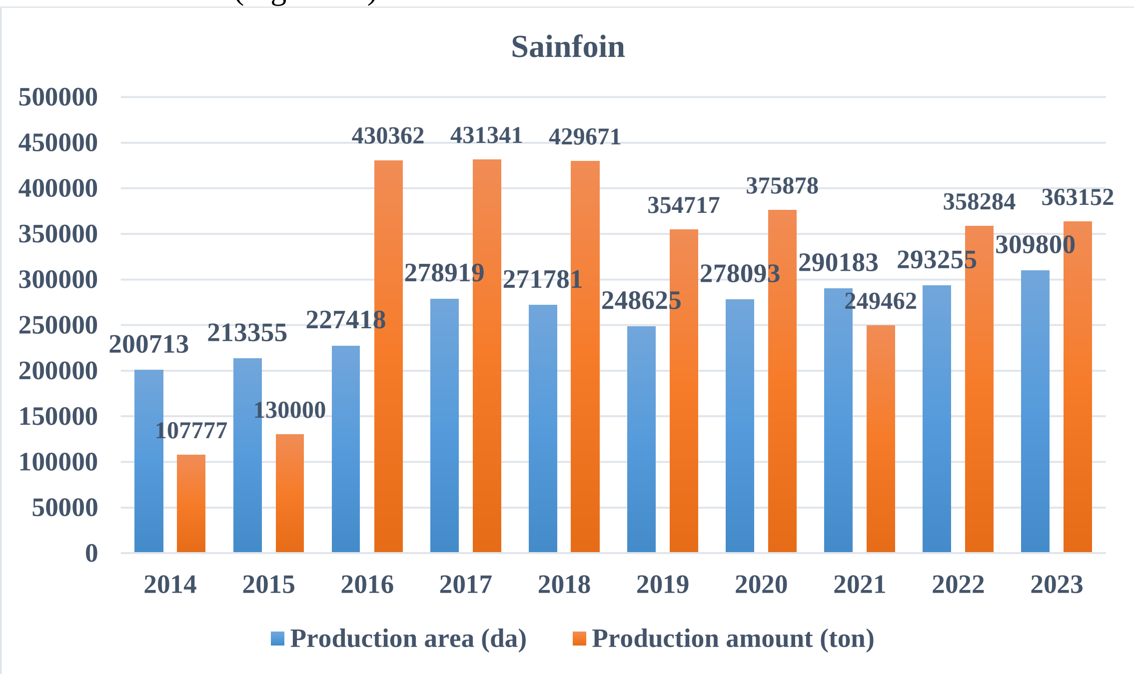


Figure 14. Sainfoin production area and production amount (TUİK 2023)

While alfalfa production was 642523 tons in 2014, it increased to 1020805 tons in 2023. In particular, alfalfa production, which reached its highest level with 2222010 tons in 2016, experienced fluctuations in the following years. While the alfalfa production area was 1048834 da in 2014, it decreased to 741549 da in 2023. The highest alfalfa production area was determined as 1042506 da in 2017. The increase in alfalfa production can be explained by the application of modern agricultural techniques and the effect of agricultural supports (Kaya and Demir, 2021). A decrease was determined in chickpea, onion and alfalfa production areas between 2014 and 2023. Although the walnut production area increased in the same time period, a decrease in the crop yield was determined (Figure 15).

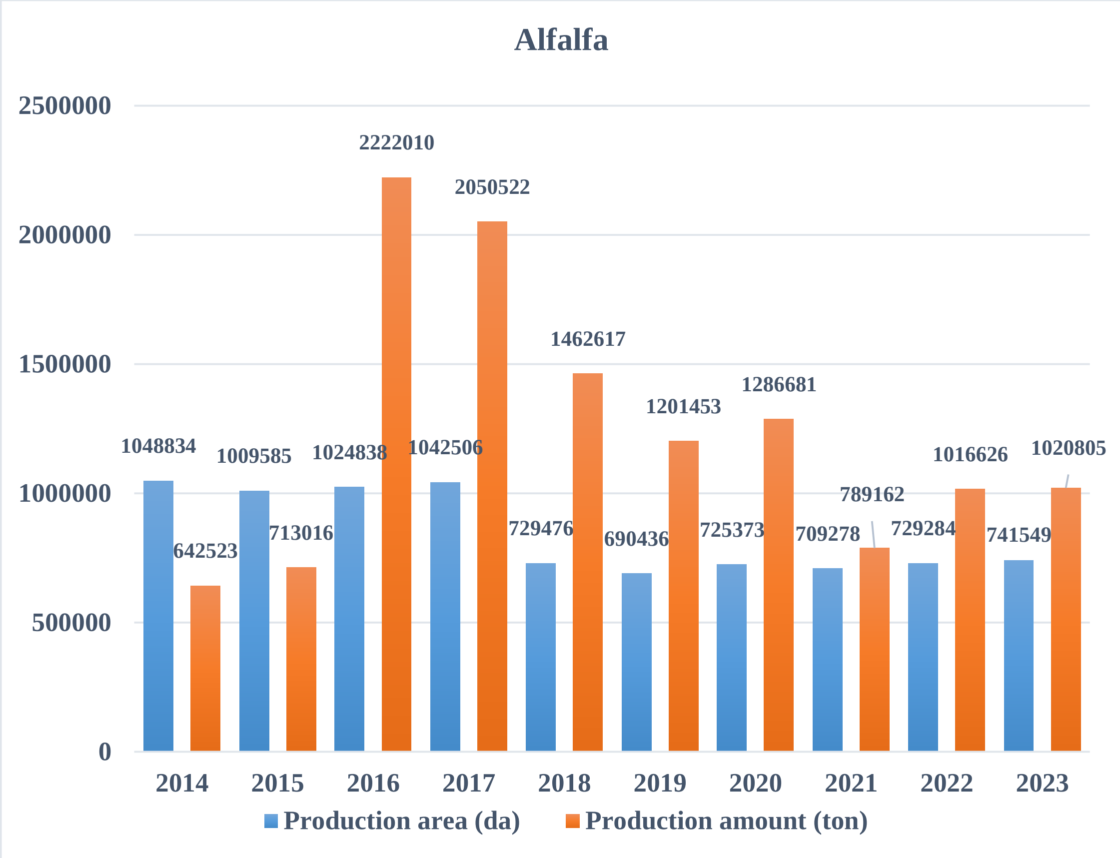


Figure 15. Alfaalfa production area and production amount (TUİK 2023)

Considering chemical fertilizer consumption, the consumption of urea, ammonium sulfate, calcium ammonium nitrate and ammonium nitrate fertilizers was 879 tons, 149 tons, 411 tons and 1243 tons, respectively, in 2014.In 2022, the consumption of these fertilizers increased and the consumption of urea, ammonium sulfate, calcium ammonium nitrate fertilizers was 1758 tons, 262 tons and 11050 tons, respectively. The consumption of diammonium phosphate fertilizer decreased from 1400 tons in 2014 to 728 tons in 2022 (Figure 16).

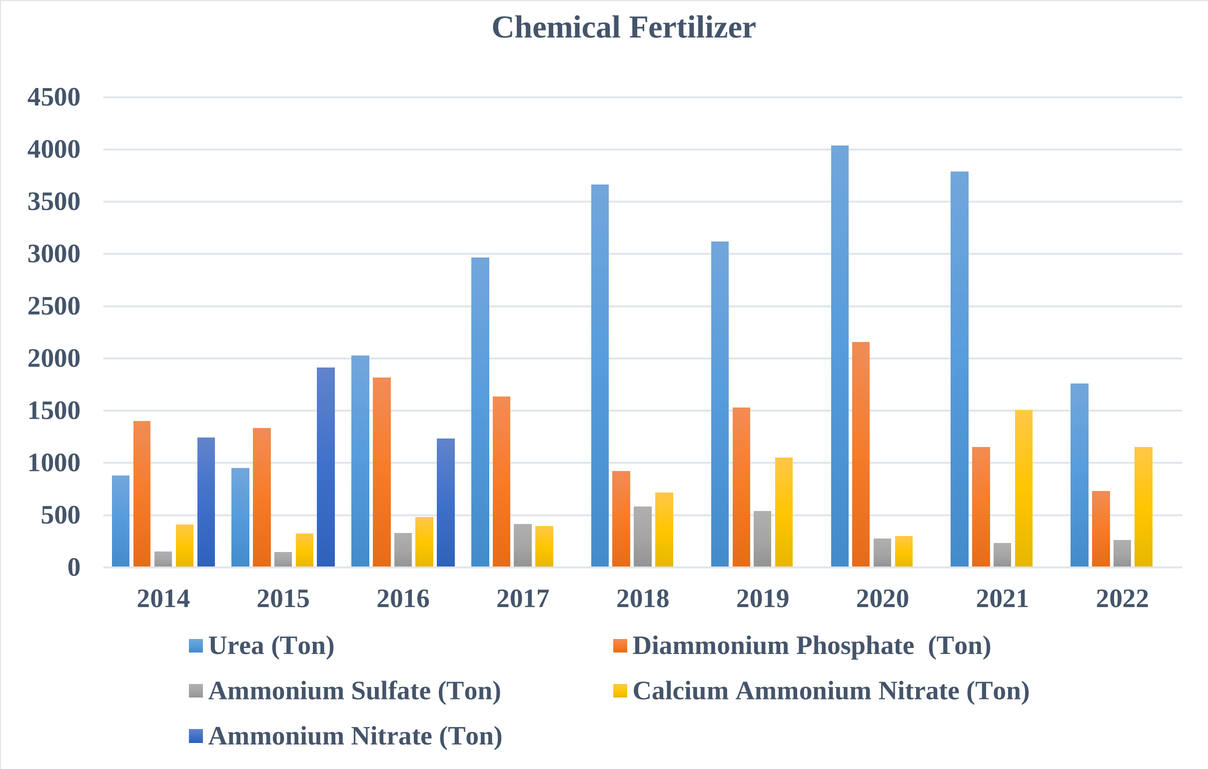


Figure 16. Amount of chemical fertilizer use between 2014-2022 (TUİK 2023)

CONCLUSIONS

In this study it was determined that crop yield belong various agricultural crops generally increased with inorganic fertilizer consumptions from 2014 to 2023 year in Van province. It has been reported that organic matter and nitrogen contents are in low level, available phosphorus level is in low or moderate level, available zinc content is in low level and lime level is high of Van Lake Basin soils by Gülser (1992).
Therefore, it can be concluded that increasing of inorganic nitrogen and phosphorus fertilizer consumptions have been effective on increasing crop yield. The fluctuations and decreases in some crop yields may be caused that differences of meteorological conditions among years.

Finez, et al. (2023) reported that excessive use of inorganic fertilizers, nutrient leaching, deterioration of physical properties of soil, accumulation of toxic chemicals in water bodies, etc. it has caused serious environmental problems and loss of biodiversity, as well as soil, air and water pollution.

Therefore, a balanced approach is needed that considers both the efficiency gains and potential environmental and health risks of using inorganic fertilizers in agricultural practices. As a result, it can be suggested that applying a fertilization program considering soil analyses results and combine applying with organic fertilizers supporting soil quality and health.

REFERENCES

Cooke, G. W. 1982.Plant Nutrition. Proceedings of the Ninth International Plant Nutrition Colloquium. Ed. A. Scaife. Slough: Commonwealth Agricultural Bureaux (1982).

Volume I, pp. 1–378, Volume II, pp. 379–750. Experimental Agriculture, 19(3), 281-281.

Finez, D. D. E., Talimbay, J. E. (2023). Advantages and Disadvantages of Using Inorganic Fertilizers for Agriculture.

Gülser, F., Karaçal, İ., (1992). Van gölü havzası büyük toprak gruplarının verimlilik durumları. YY Ü. FBE Toprak Anabilim Dalı, Yüksek Lisans Tezi.

Kaya, A., Demir, B. (2021). Modern Tarım Tekniklerinin Uygulanması ve Kaliteli Tohum Kullanımı Üzerine Etkileri. *Tarım Bilimleri Dergisi*, 34(2), 45-57.

Kumbasaroğlu, H., Aşkan, E., Dağdemir, V. (2021). Türkiye'de domatesin ekonomik analizi. Mediterranean Agricultural Sciences, 34(1), 47-54.

Mani J(2002). Early events in environmental stresses in plants: Induction mechanisms of oxidative stress. In: D. Inzè and M.V. Montague (eds.) Oxidative stress in plants. Taylor and Francis, New York. pp 217-246.

Najafi, E., Devineni, N., Khanbilvardi, R. M., & Kogan, F. (2018). Understanding the changes in global crop yields through changes in climate and technology. Earth's Future, 6(3), 410-427.

Ojeniyi, S. O., Adejobi, K. B. (2002). Effect of ash and goat dung manure on leaf nutrients composition, growth and yield of amaranthus. Nigeria Agricultural Journal, 33, 46-49.

Roy, R.N., Finck, A., Blair, G.J., Tandon, H.L.S. 2006. Plant nutrition for food security, aguide for integrated nutrient management, FAO Fertilizer and Plant Nutrition Bulletin 16,
FAO, Rome- Italy. Chapter 4. Soil fertility and crop production. 43-90, Rome, 2006.

TUİK 2023. Van Province Agricultural Product Production Data and Chemical Fertilizer Consumption Data (2014-2023). https://biruni.tuik.gov.tr/medas/?locale=tr

MEDICALLY SIGNIFICANT SPIDERS (ARACHNIDA: ARANEAE) AND HYMENOPTERANS (INSECTA: HYMENOPTERA) OF ALBANIA: A COMPREHENSIVE REVIEW OF ECOLOGY AND VENOM TOXICITY

Blerina VRENOZI[1], Arta LUGAJ[2]

[1]Research Center of Flora and Fauna, Faculty of Natural Sciences, University of Tirana, Petro Nini Luarasi 80, 1010, Tirana, Albania;
[2]Department of Biology, Faculty of Natural Sciences, University of Tirana, Boulevard "Zog I", 1001, Tirana, Albania;
Corresponding author e-mail: blerina.vrenozi@fshn.edu.al

ABSTRACT

There are several studies on spiders and insects in Albania; however, only a few papers address their venom, toxicity and medical significance. This paper presents the first comprehensive data on ecology and distribution, including their morphology and habitat preferences, as well as the venom toxicity of medically significant insects and spiders found in Albania. The data presented here has been gathered from an extensive literature review and through citizen science, focusing on social networks, written and verbal media, and observations collected from the online platform iNaturalist. Spiders from the "widow" group (genera *Latrodectus* and *Steatoda*), are frequently mentioned for the severity of their bites due to the potent neurotoxin α-latrotoxin present in their venom. On the contrary, the medical significance of hymenopterans, mainly honeybees (*Apis mellifera*), is due to the anaphylactic reactions induced by their venom. This study presents the first attempt to map the distribution of venomous spiders and insects present in Albania, altogether with detailed information on the morphology, ecology, and venom toxicity.

Keywords: Balkans, arthropods, arachnids, Hymenoptera, venomous, anaphylactic

INTRODUCTION

The subphylum Arthropoda is a diverse group of invertebrate organisms, including arachnids, insects, myriapods, and crustaceans, and account for up to 85% of all known animal species. Their venoms possess great structural diversity, and comprise proteins, peptides, and small molecules (Daly and Wilson, 2018). Most venom compounds studied so far are found in insects, scorpions and spiders. For the latter, it has been predicted that over 10 million bioactive peptides (Saez et al., 2010) are produced by the 52,340 species described so far (World Spider Catalog, 2024). The envenomation causes local and systemic symptoms associated mainly with pain (Vrenozi, 2022). However the characterization of the novel spider venom compounds has revealed therapeutic and insecticidal potential. The spiders best known in Europe for their severe envenomation belong to the family Theridiidae Sundevall, 1833 (Arachnida: Araneae). This family includes 262 species in Europe and 2537 species worldwide. Within the Theridiidae, the "true widow" of the genus *Latrodectus* Walckenaer, 1805 comprises 35 species globally and 9 in Europe (Nentwig et al., 2024; World Spider Catalog, 2024). Members of the genus *Latrodectus* are known for their medical significance and severe envenoming have been reported from black widows, the brown widows, and the redback widows (Keyler et al., 2020; Vrenozi, 2022; Weinstein, 2024). The only *Latrodectus* documented in Albania is *L. tredecimguttatus* (Rossi, 1790) (Vrenozi, 2022). The venom of some false widow spiders (genus *Steatoda*) including *Steadoda grossa* (C. L. Koch, 1838), *S. nobilis* (Thorell, 1875), *S. paykulliana* (Walckenaer, 1806), and *S. triangulosa* (Walckenaer, 1802) has been shown to be toxic to mammals (Carvajal et al., 2023; Maretić et al., 1964; Paolino et al., 2021). At least *S. paykulliana* seems to have a stronger mechanical bite than the *Latrodectus* species (Dunbar et al., 2020b; Maretić et al., 1964). *Steatoda* species documented in Albania include *S. paykulliana*, *S. triangulosa*, and *S. grossa* (Vrenozi, 2012); however, *S. nobilis* is also expected to be present based on a suspicious bitten case and a record observation (Vrenozi pers. com.). The main toxin associated with the widows' envenoming syndrome is the α-latrotoxin, a neurotoxin 15 times more potent than rattlesnake (*Crotalus atrox* Baird and Girard, 1853) venom and far more potent than cobra and coral snake venom (Thill et al., 2022). In addition to the direct toxicity of their venom, studies have shown that *S. nobilis* and *Latrodectus* sp. carry pathogenic bacteria on their body surfaces and fangs, including multidrug-resistant strains that can cause severe bacterial infection, which may develop alongside venom-driven *Latrodectus*-like symptoms. This challenges the long-held belief that spider venoms and venom systems are sterile environments (Dunbar et al., 2020b). Other medically significant but less dangerous spiders in Albania include the yellow sac spiders (family Cheiracanthiidae) *Cheiracanthium punctorium* (Villers, 1789) and *C. mildei* L. Koch, 1864 (Nentwig et al., 2024). Necrosis has been documented following envenomings by the Mediterranean brown recluse *Loxosceles rufescens* (Dufour, 1820) (Vrenozi, 2022). Bites from recluse spiders can cause similar local symptoms as *Ch. punctorium* and *Ch. mildei*, resulting in chronic pain in adults, weakness, numbness, and atypical skin lesions (Vrenozi, 2022).

While the venom of a narrow range of spiders and scorpions is the most medically important, other arthropods produce toxic venom compounds, including ants, beetles, butterflies, caterpillars, honey bees, millipedes, and wasps (Schmidt, 2019). Insects use their venom for prey capture, defence against predators, facilitating parasitism or for digestion purposes. The venom of Hymenoptera (i.e., wasps, ants, and bees) has been extensively investigated due to their medical, agricultural, and ecological importance and high toxicity (Walker et al., 2018). Several species of Hymenoptera are present in Albania, including 32 bumblebees, eight paper wasps, seven hornets, three wasps, one honeybee and 79 fire ants (Mingomataj et al., 2003; Wagner et al., 2018). Typically, bumblebees are more docile than bees or vespid wasps, so stings are much less common. The three most common species of bumblebees in Albania are *Bombus pascuorum* (Scopoli, 1763), *Bombus lucorum* (Linnaeus, 1761), and *Bombus terrestris* (Linnaeus, 1758), which are less aggressive than other insects if their nest is disturbed. Following stinging events, their venom seems to produce less allergic reactions compared to honeybees, and appears to be highly cross-reactive to their venom. Moreover, it is shown that immunotherapy with honeybee venom can protect patients with bumblebee venom allergy (Bucher, Korner, and Wüthrich, 2001). *Apis mellifera* Linnaeus, 1758 (Apidae) is the only species of honeybee present in Albania, with several breeds and hybrids. *Apis mellifera* has three common subspecies occurring in Albania, where *A. mellifera carnica* Pollmann, 1879 are found in northern and eastern Albania (Ruttner, 1988), while *A. m. macedonica* Ruttner, 1988 is present in southeast Albania, and *A. m. cecropia* Kisenwetter, 1860 is found in southern Albania (Infantidis, 1979). Its venom has distinct effects on humans, inducing local symptoms such as pain, redness, and swelling, alongside systemic symptoms affecting the respiratory tract, cardiovascular, and gastrointestinal system, up to eliciting allergic reactions (Bilo and Bonifazi, 2009). Hornets (family Vespidae) are usually larger than most wasps and are known for their ability to deliver more painful stings; however, they also differ in nest type and aggressiveness (Mingomataj et al., 2003). The oriental hornet wasp, *Vespa orientalis* Linnaeus, 1761, is a social insect present in Albania with a public health concern due to their size and aggressiveness as they can inflict multiple, painful stings. Only females have stingers, and workers use these to defend themselves and the colony. This species has strong jaws, which are effective for digging and defence (Cetkovic, 2003). Paper wasps (family Vespidae), of which *Polistes dominula* (Krishti, 1791) is one of the most common and locally well-known species, are present in Albania and are known for building nests made of a paper-like material, which they produce by chewing plant fibres mixed with saliva (Bagriacik, 2012). Hymenopterans with less allergic reactions of their venom are the ants. Fire ants (Formicidae: *Solenopsis*) are known for their unique and painful stinging process, different from bees and wasps as they are capable of stinging multiple times without losing their stinger, causing a burning sensation rather than a typical protein-based sting, due to the alkaloids that is the main component of their venom. Their vernacular name finds its origins in the intense burning sensation that usually follows their stings (Greenberg, 2009). The sting from fire ants can cause unpleasant symptoms and may lead to allergic reactions. *Solenopsis fugax* Latreille, 1798, is a small Myrmicinae ant (1.2-1.5 mm) occurring in Albania.

METHODOLOGY

A systematic search was performed, screening the literature and information on the medically significant spiders and insects in Albania. Google Scholar (https://scholar.google.com/) is mainly used for the literature scanning; however, literature is scanned also from PubMed (https://pubmed.ncbi.nlm.nih.gov/). Several keywords were used to extract papers, some of which are: spiders of Albania, venomous spiders, black widow Albania, black widow bite, black widow envenoming, spider venom toxicity, arachnids and insect venom, araneae, hymenoptera of Albania, hymenoptera reaction, bumblebee sting, distribution of wasp, allergic reaction from honeybee, the venom of *V. orientalis*, morphological features of hornets etc. The observations in the citizen science website, iNaturalist (https://www.inaturalist.org), are scanned for the selected country of Albania using either the name of each species of spiders and insects, or their genus. Users record observations gathered as part of citizen science projects, school projects, or simply personal observations. Observations are uploaded to a publicly accessible "Community ID" system, and community users can suggest taxonomic identifications. However, not all the observations are considered, as we observed suspicious morphological features for the exact identification. Some iNaturalist data is also part of another website, GBIF, which provides information from the datasets of the scientific collections. GBIF which was scanned for the country of Albania using the keywords of the species names of the medically significant spiders and insects known so far in Albania. The verbal and written media including TV interviews from toxicologists and journals in Albanian language (a2news, Balkan web, Lajme.rtsh.al, Shqiptarja.com, Syri TV, ABC news Albania, RTV Klan), the social network of Facebook (https://www.facebook.com/), are checked for the observations in the Facebook home, public Facebook group "Electronic Register of Albanian Species [Regjistri Elektronik i Specieve Shqiptare]", using keywords in Albanian language in brackets, such as: spider [merimanga], black spider [merimanga e zezë], black widow [vejusha e zezë], venomous spider [merimanga helmuese], spiders bites [pickime të merimangave], insects [insekte], honeybee [bletë], wasp [grerëz], ants [milingona], etc. The Latin names of the medically significant spiders and insect species were also used as keywords to search for their records.

The information is extracted from a total of 96 papers, two spider websites (Nentwig et al., 2024; World Spider Catalog, 2024), from citizen science observations (iNaturalist, GBIF), mass media communication (written and verbal media), and from personal observation of the first author.

RESULTS and DISCUSSION

1. The information from the citizen science and mass media communication

The ability of citizens to access the latest information about the risks and management of spider and insect bites may contribute to prompt and responsible action, reducing the incidence of severe reactions and improving overall public health outcomes. Over the past two decades, the development of citizen science platforms has significantly increased public enthusiasm for observing and documenting species occurrence and interactions between organisms. This growing interest has led to the production of large amounts of biodiversity-related data at unprecedented temporal and spatial scales, rendering data of tremendous value to the scientific community (Bonney et al., 2014; Cox et al., 2015; Kittelberger et al., 2021). Recently, the scientific community has increasingly recognized the value of citizen-driven scientific data (Adler et al., 2020). The iNaturalist, established in 2008, is a global network that functions as an online social network and identification system for amateur naturalists based on peer assessment of species identification (Seltzer, 2019). By selecting a taxon in iNaturalist, users can view images, dates, and coordinates, with an accurate interactive distribution map for each taxon observation. The citizen science website iNaturalist is available for species data occurrence, where amateurs have added locality, date, photo, and species name information; while another website, GBIF - the Global Biodiversity Information Facility (https://www.gbif.org/), a noteworthy global repository of biodata with a vast network of users and entities, primarily describe museum collections that publish datasets such as natural history museums, institutions responsible for collections such as universities or research centres, and researchers from these institutions.

Mass media communication (newspapers, television, and social media) is an integral part of society and influences many aspects of human life. It is used to disseminate information for the vast majority of the public audience with a tendency to exaggerate findings, presenting them in sensationalist terms to simplify, and to avoid complex issues (Brechman et al., 2009; Nelkin, 1995). Social media provides citizen science data, documenting species occurrences of wild animals, and mainly Facebook (launched in 2004), the largest network in the world, stores many images altogether and overlapping information with other socials such as Instagram, LinkedIn and Twitter (Anderson et al., 2012; Lister, 2017). Facebook offers the possibility to create specialised groups or pages in which the administrators can help in identifying taxa photographed in the field (Chamberlain, 2018), giving only the name of the location but without geotagging of the posts. Valuable information from the verbal and written media about cases of spider and hymenopteran bites in Albania, rely on the data about their toxicity. However, the information conveyed is not always correct, and therefore further emulates the fear of these species. The media framing of severe envenoming cases (sometimes fatal) due to venomous spiders has alarmed habitants of the areas concerned, leading to strong emotional reactions when a spider is seen, whether it is medically significant or not (Mammola et al., 2020). Mass media communication has played a significant role in the dissemination of information on envenoming cases and the occurrence of venomous organisms in Albania. These data accomplish the citizen science information, where individuals, amateurs and specialists, provide important data on different species (Table 1).

2. Spiders envenoming in Albania

2.1 Morphology and identification criteria

Spiders often display a wide variety of habitats and different body colours. The false widow spider, *S. paykulliana* is often mistaken by amateurs in the social networks for the black widow spider, based on the similar round, shiny, uniformly black abdomen (Nentwig et al., 2024). Instead, the red pattern surrounding the dorsal black abdomen distinguishes *S. paykulliana* from adult females of the black widow spider. *L. tredecimguttatus* has morphological variations not only among various development phases from juveniles to adults, but also among the adults. The typical 13 orange or red patterns on their brownish to blackish abdomen are more consistent for juveniles and subadults (Levy and Amitai, 1983) (Fig. 1B), whereas adults of *L. tredecimguttatus* usually have only one antero-posterior red pattern close to the spinnerets at the end of the round shiny black abdomen (Vrenozi pers. com) (Fig. 1C), and in other cases can be completely black as observed in France (Dugon pers. com.), Israel (Shulov and Weissman, 1959), Iran (Shafaie et al., 2021). Contrarily, *S. paykulliana* has only a red transversal line crossing the antero-dorsal aspect of the black, round, shiny abdomen close to the cephalothorax (Fig. 1G). Other medically significant theridiid spiders of the genus *Steatoda* occurring in Albania have a highly variable body colour from yellow-white to brown with a dorsal pattern, to almost black with a faded dorsal pattern (*S. grossa*) (Fig. 1E), or females purple-brown with white stripes and males beige and white with dark purple folium pattern and four larger reddish impressed spots (*S. nobilis*) (Fig. 1F), to yellowish patterns that sometimes are broken into separate white spots on a brown to black abdomen (*S. triangulosa*) (Fig. 1H). The yellow sac spiders *Ch. punctorium* (Fig. 1A) and *Ch. mildei* have similar coloration with a pale yellow-green abdomen and a dark cardiac mark. In contrast, the Mediterranean brown recluse *L. rufescens* has a brown coloration (Nentwig et al., 2024) (Fig. 1D).

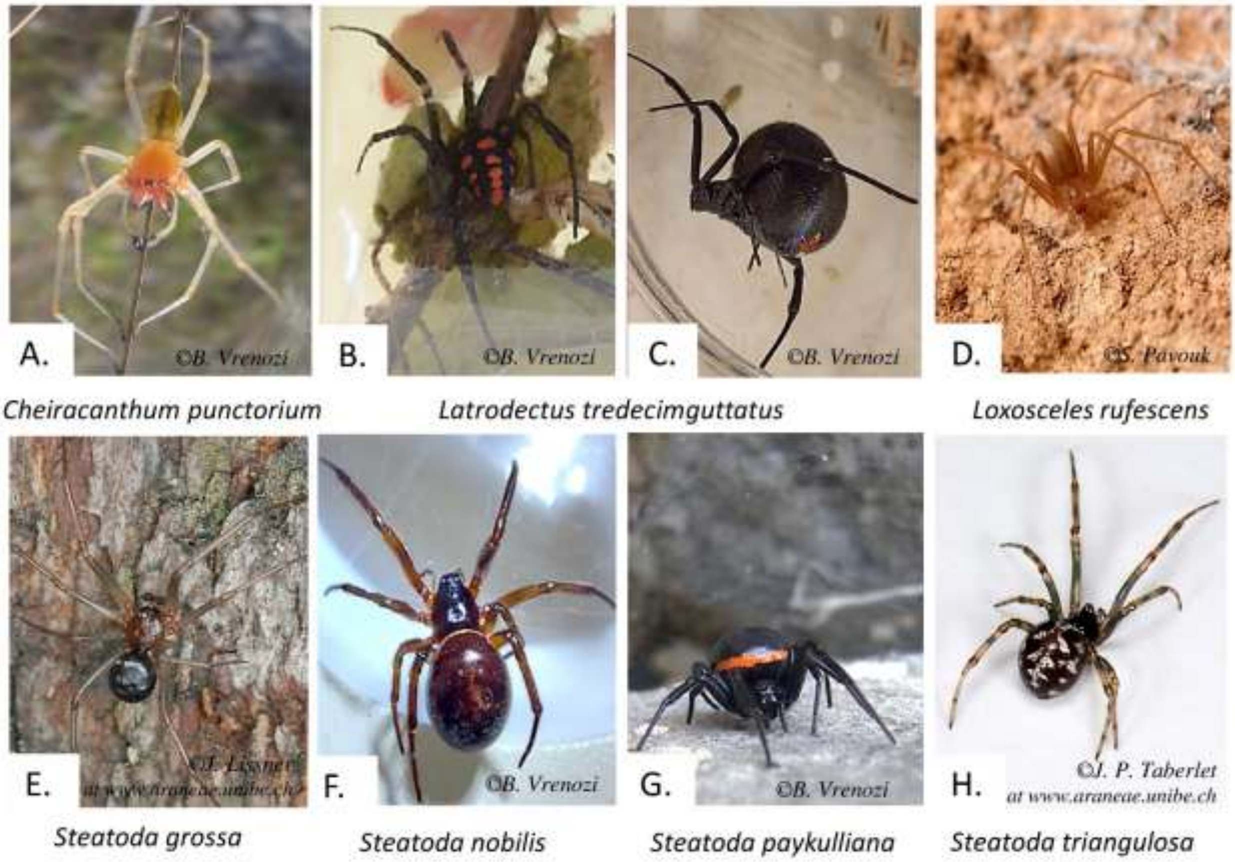


Figure 1. Venomous spiders: **A**– *Cheiracanthium punctorium*, **B** & **C**– *Latrodectus tredecimguttatus*, **D**– *Loxosceles rufescens*, **E**– *Steatoda grossa*, **F**– *S. nobilis*, **G**– *S. paykulliana*, **H**– *S. triangulosa*

2.2 Spider bites, prevalence and activities associated with envenoming

The venom of Theridiid spiders has mostly evolved to paralyse or kill their arthropod prey, although toxicity toward vertebrates may have evolved as a defensive behaviour against predators and as response to accidental crushing (Rohou et al., 2007). Within this paradigm, widow spiders are not aggressive toward humans, and envenomings occur only when the spider feels threatened. Bites from *Latrodectus* sp. typically occur from females, since they are much larger than males (7-15 mm versus 4-7 mm). Additionally, males are not thought to have the ability to break human skin with their small chelicerae (Nentwig et al., 2024; Peterson, 2006). Considering the small size of widow spiders, only a minute amount of the injected venom, makes it rarely fatal to humans. Moreover, the black widow spider venom is known to kill small mammals in captivity. Thus, the lethal dose of the black widow spiders (*L. tredecimguttatus*) is calculated as 0.013 mg venom per mouse, translated to an overall LD50 of 0.9 mg/kg mouse, with estimation that venom of one spider has enough potency to kill 40 mice (Bettini and Maroli, 1978). During the envenomation, the average amount that the black widows injects to the target, depending on the pressure on spider's legs and body, is respectively between 0.0346 - 0.11 mg of the available venom in a single bite (*L. hesperus*) (Nelsen et al., 2014). Since venom effect tends to be dose-dependent, younger victims with relatively smaller body sizes are more at risk of facing severe symptoms. Recent reports on black widow spider envenomation in Albania have reported 125 patients from 2009 to 2018, where 7.2% of which had severe systemic symptoms (Vrenozi, 2022). The venom itself is physiologically expensive, and spiders are thought to use it carefully (Wigger et al., 2002). This may explain the variations in envenoming symptoms, and the frequent occurrence of "dry bites" during which no venom is injected at all. Some of the more common severe symptoms reported from widow bites may be explained by panic attacks and anxiety resulting from the fear of spiders (i.e, arachnophobia), a phobia that social media have contributed to, since they often provide a distorted perception of the spider bite risk (Mammola et al., 2020). Various documented cases of false widow spider bites of *S. nobilis* have revealed the severity of their venom in bitten patients, which is attributed to the presence of α-latrotoxin, a 'vertebrate-specific' neurotoxin (Dunbar et al., 2018; Dunbar et al., 2020a; Dunbar et al., 2022; Warrell et al., 1991). Another false widow spider, *S. triangulosa*, has been documented to feed on small vertebrates, first immobilizing and wrapping them in silk, then paralyzing them and causing their death, respectively, 10 and 20 minutes after the bite (Vitkauskaite et al., 2021). Therefore, *L. tredecimguttatus* and the false widow spiders are considered medically significant species and a growing threat to public health, especially for patients of small size (Vrenozi, 2022). However, we should distinguish the venom neurotoxicity from the post-bite bacterial infections found on the chelicerae of *S. nobilis*. The infection is most likely to be the result of spider-borne bacterial zoonosis rather than opportunistic infections by commensal skin bacteria. This can cause sepsis which in turn can, in very rare cases, leads to paralysis or amputation (Ahrens and Crocker, 2011; Dunbar et al., 2020b). In addition to neurotoxins, spider venom can have a cytotoxic effect, such as in the case of the brown recluse, *L. rufescens*, which causes necrosis in the bite area (Vrenozi, 2022). Necrosis can leave a post-bite mark, which would require plastic surgery as the ultimate hospitalization phase of an already serious case. Otherwise there is no need to hospitalize bitten patients. Cases of bites from *Ch. punctorium* and *Ch. mildei* are rare, do not require hospitalization, and occur during outdoor activities, since the spider is nocturnal and tends to reside far from inhabited areas. The bite results in an immediate burning sensation reaching maximum intensity after 5-20 minutes, with cutaneous effects of local redness, swelling, itching, numbness, and neurological effects such as insomnia. The local symptoms disappear within 2 hours in adults, except for paraesthesia at the bite site. In children, the pain may persist over 12 hours but complete recovery is expected after three to four days; there is no conclusive association of *Cheiracanthium* envenomings with necrosis (Papini, 2012).

2.3 Habitat preferences and distribution map of venomous spiders in Albania

The growing number of human cases highlights the importance of public awareness and the role of technology in using social media and sharing information to help people take necessary precautions, although spiders are usually mistaken for insects in the media. Moreover, based on misinformation and fear spread from mass media (Mammola et al., 2020), victims usually refer to all cases of insect stings and spider bites as being spider envenoming. Spider bites are a concern, especially during the summer, when temperatures are high. This is further enhanced by the recent increase in temperatures due to global climate change and resulting local heat waves (Vrenozi, 2022). There are eight species of spider with potential medical significance in Albania. However, only envenomings by the black widow *L. tredecimguttatus* might require hospitalisation in Albania. All other spider bites require observation of the bite site and the use of topical treatments (Vrenozi, 2022). In very rare cases hospitalisation might be required due to bacterial infections that might lead to sepsis (as reported for *S. nobilis*, (Dunbar et al., 2020b). During the summer period, various black widow spider bites have occurred and several individual were observed in different regions of Albania, mainly in Berat, Elbasan, Fier, Lushnjë, Rrogozhinë, Tepelenë (Balkan web, Facebook community, iNaturalist community, InFormim.al, Infoweb, lajme.rtsh.al, Lushnja.info, RTK 4+, Shqiptarja.com, Syri TV). Here are found the typical habitats of black widow spiders, such as cultivated farmlands with wheat, been and other crops, but also uncultivated lands as dry habitats or meadows, dunes, sandy beaches, forests with fallen trees, where *L. tredecimguttatus* build their webs on the bases of the lower vegetation under the leaves, stones or fallen trees (Bettini and Maroli, 1978; Vrenozi, 2022). The municipality of Divjaka is the main area in Albania where the black widow spider has the largest numbers of observations and spider bites reported from hospitalised cases (Vrenozi, 2022), and mass media and citizen science where two fatal cases are mentioned in the Bedat village (Facebook community, iNaturalist community, lajme.rtsh.al, Lushnja.info). The area has the most important agriculture in the region, where main products are potatoes, watermelons, and other vegetables that reach national and international markets, and also beans, maize, wheat and other crops for home consumption (Canali et al., 1998). However, there are other observations in the south-eastern villages of Vrinë and Leskovik in the municipalities of Konispol and Korcë respectively (Kůrka et al., 2020) (Table 1) (Figure 2A).

The *Steatoda* species are usually found on and inside buildings, on fences, cellars, caves, cracks in stone walls, and tree trunks (Dugon et al., 2017; Kovács and Szinetár, 2018). Two suspected bitten cases are observed in the suburban areas in the Tirana district (Fresk and Kombinat), with a typical wound of *S. nobilis* with necrosis and infection that in one case had required surgical intervention (Vrenozi pers. com). In addition one field observation of a female individual is posted on Facebook in the rocky area of the village Kaninë in Vlorë municipality. Whereas *S. paykulliana* is rarely encountered in orchards and meadows, and prefers arid and semi-arid habitats, such as dry grasslands, rock crevices, limestones, and stony hills (Nentwig et al., 2024; Vrenozi, 2022). This spider is often mistaken for a black widow due to the black round abdomen in the iNaturalist community and mass media. The distribution area is wider and includes habitats from northern (Dibër and Pukë) to southern Albania (Sarandë). It is based mainly in faunistic papers (Caporiacco, 1932, 1949; Deltshev et al., 2011; Kůrka et al., 2020; Strand, 1919; van Helsdingen and IJland, 2015; Vrenozi, 2012; Vrenozi and Dunlop, 2013; Vrenozi and Haxhiu, 2008; Vrenozi and Jäger, 2013), in iNaturalist community, GBIF.org, and unpublished data of the first author. Recent years (2021, 2023), the false widow spiders of *S. grossa* have been observed in the municipality of Durrës in Albania (GBIF.org), but there are no reported cases of hospitalized patients in Albania. The less significant spider of the false widows, *S. triangulosa*, has a wider distribution inhabiting mainly urban localities, on the walls, stony habitats or lower vegetation (Durrës, Fier, Gjirokastër, Përmet, Sarandë, Vlorë) as observed in iNaturalist community, GBIF.org and several papers (Deltshev et al., 2011; iNaturalist; Naumova, 2020; van Helsdingen and IJland, 2015; Vrenozi, 2012; Vrenozi and Haxhiu, 2008) (Table 1) (Figure 2A).

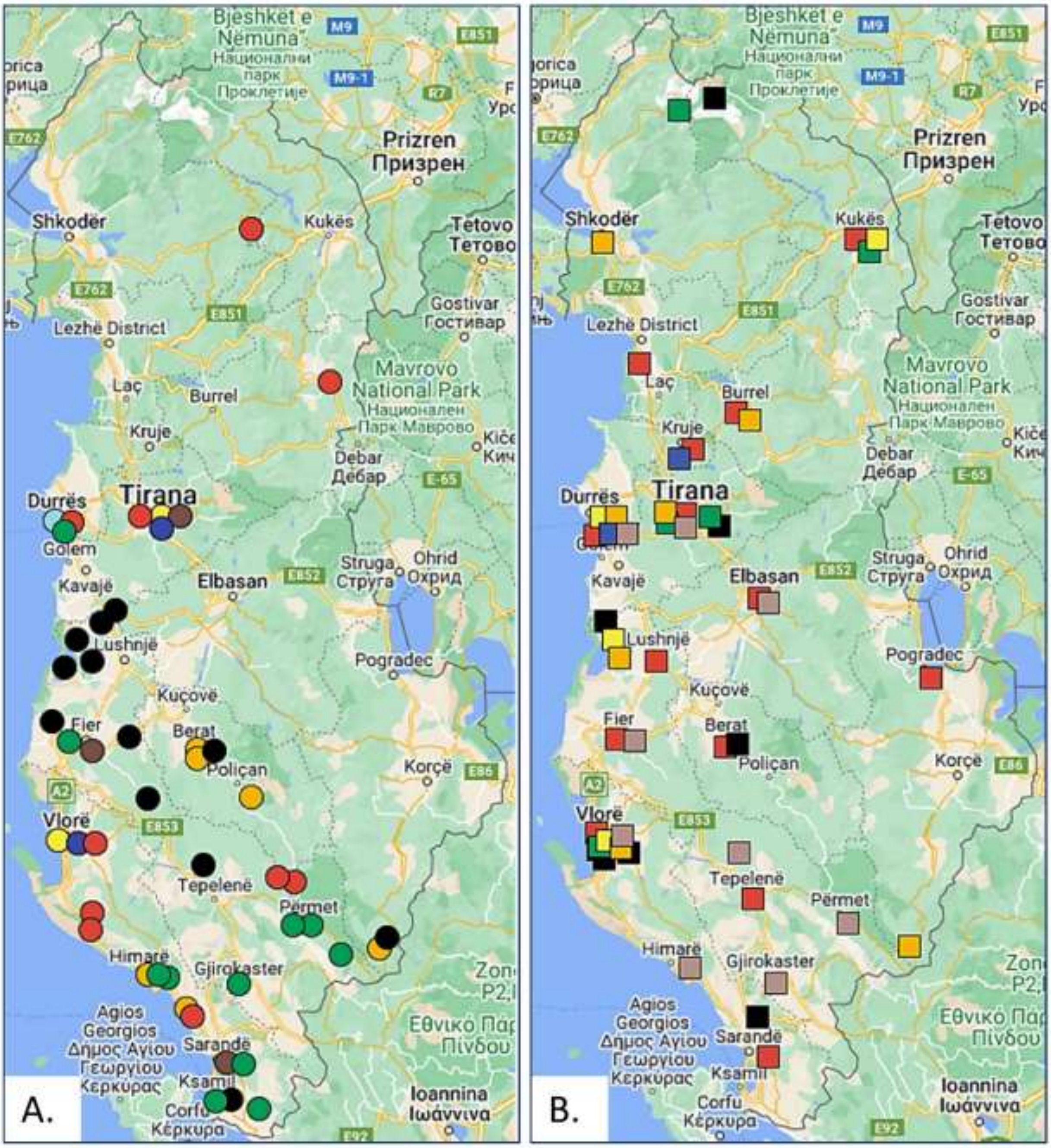


Figure 2. Distribution maps (google.com/maps): **A**— Venomous spiders (circles): *Cheiracanthium punctorium* (yellow), *Ch. Mildei* (orange), *Latrodectus tredecimguttatus* (black), *Loxosceles rufescens* (brown), *Steatoda grossa* (light blue), *S. nobilis* (dark blue), *S. paykulliana* (red), *S. triangulosa* (green). **B**—Venomous insects (quadrates): *Apis mellifera* (red), *Bombus lucorum* (black), *B. pascuorum* (green), *B. terrestris* (yellow), *Polistes dominula* (orange), *Solenopsis fugax* (blue), *Vespa orientalis* (brown)

The brown Mediterranean spider is found in a variety of natural habitats such as dry, under stones, rock crevices, but is widely distributed as a house spider as it is found in and around houses and buildings, making this species easy to transport in vehicles in other countries. This makes *L. rufescens* bites occurring frequently indoors while dressing up and sleeping (Gertsch and Ennik, 1983; Vrenozi, 2022). In Albania, there is reported the necrosis wound of the post bitten cases within the apartment in municipality of Fier, and two observations within a toilette in the city of Tiranë and under stones in Sarandë (Naumova and Deltshev, 2021; Vrenozi, 2022). In contrast, *Ch. punctorium* and *Ch. mildei* inhabit shrubs and trees in warm, open habitats. Consequently, sac spider bite cases are rare, typically occurring during outdoor activities (Hörweg, 2023). Considering that the species can be mistaken with other similar species of the same genus, findings in Albania are based mainly on the faunistic papers (Deltshev et al., 2011; Kůrka et al., 2020; van Helsdingen and IJland, 2015; Vrenozi, 2012; Vrenozi and Haxhiu, 2008), and on one confirmed observation on GBIF.org and the B. Vrenozi pers.com (Table 1) (Figure 2A).

3. Hymenopterans envenoming in Albania

3.1 Morphology and identification criteria

Hymenoptera is a large order of insects and they can be distinguished from other insects by the presence of the waist between the thorax and abdomen, antennae, wings, mouthparts, metamorphosis, sting and social structure, used as identification criteria (Schmidt and Smith, 2006). The common bumblebee, *B. pascuorum* has a yellow or reddish-brown thorax, while the hairs of the first four abdominal segments are grey and the hairs of the fifth and sixth segments are yellow or reddish-brown (Lecocq et al., 2015) (Fig. 3C). The most common large European ground-dwelling bumblebee, *B. terrestris*, is a Mediterranean species known as the owl-tailed bumblebee. Workers of *B. terrestris* have white-bottomed abdomens and darker yellow bands (Fig. 3D) compared to those of *B. lucorum*, that are characterised by a lemon-yellow collar behind the head, a bright yellow band in the middle of the body, and a pure white tail (Bertsch et al., 2004). There exists breeding data for ten subspecies of honey bees *Apis mellifer*a (Fig. 3A) in Albania, as well as hybrid races of several breeds known as Buckfast bees (Kuliçi et al., 2023). Three subspecies are native in Albania, *A. m. carnica*, *A. m. macedonica*, and *A. m. cecropia*: *A. m. carnica*, has a generally dark body colour with a grey tinge, ventral stripes characterized by a lighter grey or brown, short hair that is usually grey compared to other species, and a very high elbow joint; *A. m. macedonica* usually has a dark body with light grey or brown abdominal stripes, short abdomen, and long legs; *A. m. cecropia* has a golden brown coloration, slightly darker than the other honeybees (Gregori and Starman, 2003). *Vespa orientalis* has a reddish-brown colour with thick yellow stripes on the abdomen and characteristic yellow spots between the eyes (Cetkovic, 2003) (Fig. 3G). *P. dominula* differs from other wasp species due to its specific body length, wing dimensions, and distinctive black and yellow coloration. Adult paper wasps have a black and yellow body, several triangular eye-like spots on the dorsal aspect of the thorax and yellow antennae. *P. dominula* possesses a yellow postocular stripe, often narrowed, and the female's mandible is black, sometimes with yellow spots (Tibbetts et al., 2011) (Fig. 3E). The small Myrmicinae ant, *S. fugax*, is a native species in Europe known for its reddish-brown color and their ability to sting in an aggressive behaviour (Fig. 3F).

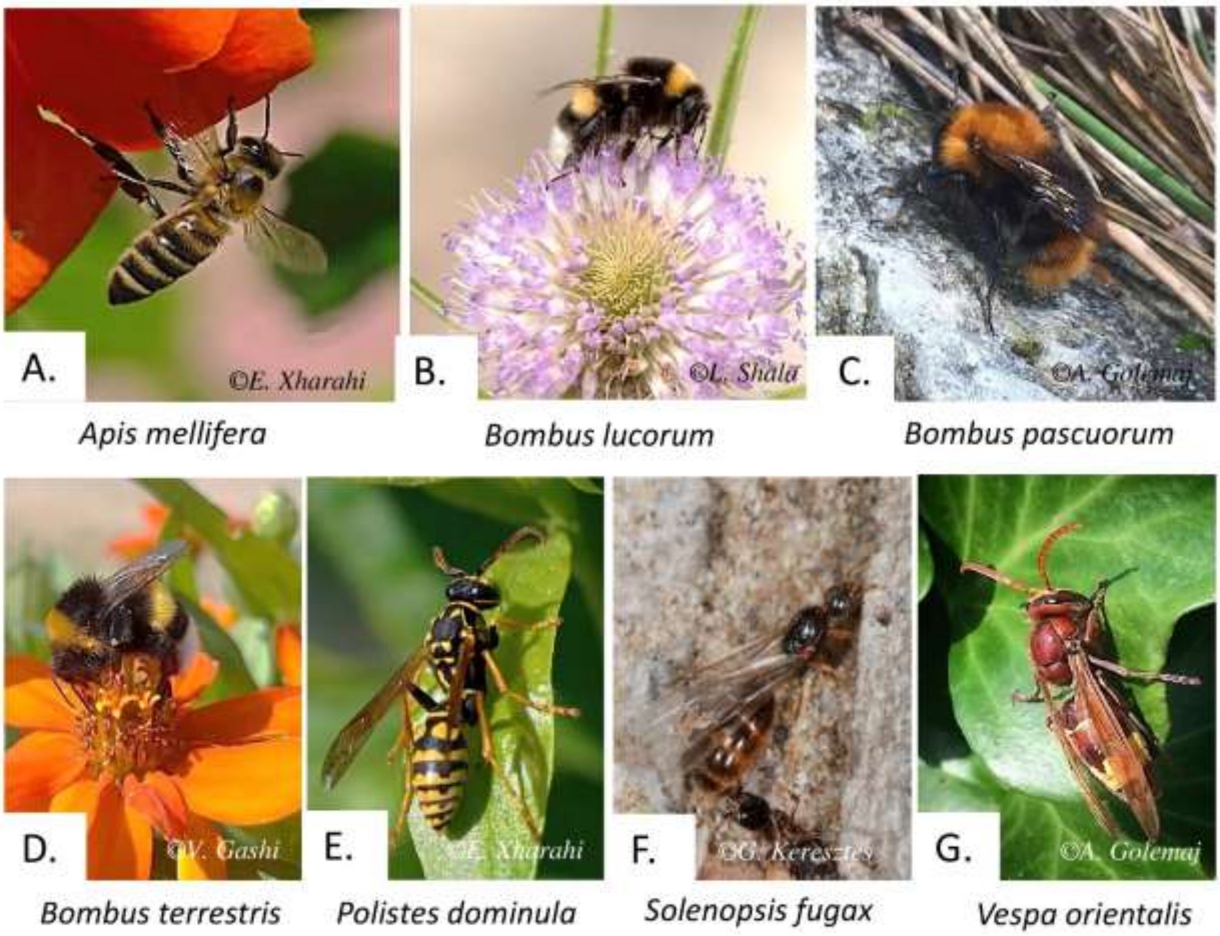


Figure 3. Venomous insects: **A**– *Apis mellifera*, **B**– *Bombus lucorum*, **C**– *B. pascuorum*, **D**– *B. terrestris*, **E**– *Polistes dominula*, **F**– *Solenopsis fugax*, **G**– *Vespa orientalis*

3.2 Hymenopterans stings, prevalence and activities associated with envenoming

Hymenopteran venom is usually stored in reservoirs within the insect's body and can be injected into the victim through an actual sting. Hymenopterans toxins consist of various chemicals or complex combinations of bioactive compounds, including small molecules, polyamines, and peptide toxins that interact with cell membrane receptors to provoke allergic reactions (Mingomataj et al., 2003). Hymenoptera venom allergy is an epidemiologically underestimated condition causing morbidity worldwide. Severe anaphylactic shocks following the stings from honeybees (*A. mellifera*) and wasps can lead to cerebral or myocardial ischemia due to the presence of numerous protein allergens, most of which have enzymatic activities, such as phospholipase A2, hyaluronidase, acid phosphatase and non-enzymatic proteins including melittin, which can trigger strong allergic reactions in sensitive individuals (Burzyńska and Piasecka-Kwiatkowska, 2021; Golden, 2007). We can mention several honeybee reactions such as the appearance of erythema, pruritus, urticaria, and angioedema on the skin; myocardial depression, hypotension, and shock in the cardiovascular system; edema of the larynx and bronchospasm in the respiratory tract; and in the gastrointestinal system the predominant symptoms are nausea, vomiting, and fecal incontinence (Bilo and Bonifazi, 2009). The European honeybee, *A. mellifera*, is the most common species of bee involved in stings and envenomation. Twelve allergens have been identified from its venom so far. *A. mellifera* die after stinging as the barbed stinger with parts of their abdomen remains in the target. This is in sharp contrast with wasps, hornets, and ants, all of which can potentially inflict repeated stings without dying (Fitzgerald and Flood, 2006). Both honeybees and wasps produce one common allergen with hyaluronidase activity that helps spread venom by destroying hyaluronic acid within the skin. Species also possess unique phospholipase-based venom allergens that play a crucial role in breaking down cell membranes and increasing the spread and effect of the venom. Fire ants and vespids possess at least one allergen in common, antigen 5, with unknown biological activity (King and Spangfort, 2000). The venom of *B. pascuorum* have a direct and immediate effect on physiological processes, causing paralysis and instant pain, owing to the presence of different allergenic elements within the venom profile (Casewell et al., 2013). Unlike honeybees, bumblebees can sting multiple times without attachment of the sting apparatus to the skin. The protein content per bumblebee sting is 10–31 μg depending on the species compared with 59 μg per honey bee sting. Allergic reactions to bumble bee stings are much less common than allergic reactions to honeybee stings, and their venom appears to be chemically and antigenically related (Hoffman and Jacobson, 1996). As bumblebees are not aggressive, the risk of being stung is very low in the general population. The worldwide use of domesticated bumblebees as crop pollinators has led to an increasing prevalence of bumblebee venom allergy, especially in greenhouse workers and bumblebee farm employees (Bucher et al., 2001). The stings of female *B. lucorum* can cause localized pain, swelling, and redness due to the mild neurotoxins present in their venom. Symptoms usually are transient, but serious complications such as anaphylaxis, angioedema and toxic reactions could arise without proper treatment. In humans, the bite may result in pain, swelling, and sometimes allergic reactions, but not life-threatening (Bucher et al., 2001). The venom of *V. orientalis* primarily poses a health risk to humans in the form of allergic reactions due to the antihistamine-releasing activity of Mastoparan O, which can contribute to allergic reactions. Usually, stings result in severe pain, local irritation, and urticarial papules (Nakajima et al., 1985). In some cases, individuals may experience more severe symptoms, such as extensive swelling, difficulty breathing, a drop in blood pressure, and anaphylaxis, especially in those who are allergic to wasp stings. Effective treatment can include the administration of antihistamines, pain relievers, and, in severe cases, epinephrine to counteract anaphylactic reactions (Mukund and Gawade, 2011). *P. dominula* and *Vespula* spp. are closely related to allergy-eliciting Hymenoptera species. Stings by the paper wasp *P. dominula* often result in allergic reactions and appears to be potentially cross-reactive, due to their venom allergens composed of homologous proteins present in other wasp venoms too (Grosch et al., 2020; 2021). Fire ants are known for their distinctive and painful stinging process that can cause a burning sensation, swelling, redness and itching. A burning sensation can last a few minutes and the itching can last up to a week. The skin eruption can be completely healed in 2 weeks. They can have a slow venom injection, and the onset of pain from a fire ant sting generally is delayed (Romita et al., 2018). This delay can sometimes prevent an immediate reaction to the sting, potentially allowing the ant to deliver multiple stings. The clinical effects caused by ant stings are associated with clinical manifestations ranging from localized reactions such as pain, papules, vesicles, pustules, and ulcers to systemic reactions with blurred vision, dizziness, vomiting, diarrhea, and anaphylaxis. *S. fugax* has powerful mandibles and retractable sting, which delivers irritant alkaloid venom. Envenoming of human beings by *S. fugax* have reported the most common local symptoms that include severe itching of papular-purpuric eruptions at the location of the stings (Romita et al., 2018; Stingeni et al., 2002). Its venom can cause significant local or systemic reactions, up to fatal cases in humans due to multisystem toxicity in case of multiple stings (Korman et al., 1990), although their sting is mild due to their small size (Graham et al., 2004).

Based on the documented cases in Albania, related to the hospitalized patients from 1987 to 1996, approximately 111 human cases were reported in Albania from patients who were diagnosed with reactions to Hymenoptera sting venom (Mingomataj et al., 2003), and 37 patients with a mean age of 28 years received stings from Hymenoptera, respectively: 59.5% from honeybees, 5.4% from paper wasps and 35.1% from bumblebees (Mingomataj et al., 2002).

3.3 Habitat preferences and distribution map of venomous hymenopterans in Albania

The reported medically significant insect cases in citizen science are not for their bites, but mainly from observation in nature (iNaturalist community, GBIF.org, Facebook community), and partly published in faunistic papers (Cetkovic 2003; Dedej et al., 1996; Mingomataj et al. 2002, 2003) and mass media communication (a2news). Hymenopterans are found in a wide variety of habitats all over Albania. Bumble bees are adapted to a diversity of climates and habitats, and are active even when light intensity is low. They are able to continue foraging even at temperatures as low as 10°C and as high as 32°C, because of their relatively large body sizes and dense pile. Bumblebees (Apidae) are known for their robust body and distinctive, loud buzzing sound when flying; they are larger, hairier, and present more colour variations compared to bees (Apidae) (Bucher et al., 2001). The bumblebee, *B. pascuorum*, is commonly found in the northern Albanian Alps (Shkodër and Kukës), in a wide range of habitats including pastures, meadows, in urban areas, wasteland, near waterways and in forests (Tiranë and Vlorë) (Plowright et al., 1997) (Fig. 2B). This is the closest relative of honeybees and differs among them as they have unique characteristics, such as adaptation to colder climates allowing them to live at higher latitudes and altitudes even when light intensity is low. This ability is attributed to their unique thermoregulatory properties, allowing them to tolerate various climatic conditions while being active even in winter (Pradervand et al., 2011). *B. terrestris* is known for its wide distribution, large colony production, and adaptability to artificial conditions (Velthuis and Van Doorn, 2006). The optimum temperature of *B. terrestris* is 32°C. As they prefer warm climates, they are present in the western areas of Albania (Durrës, Lushnje and Vlorë) and are a native species in the littoral districts of Durrës, Kavaja, and Vlorë (Rasmont et al., 2008). However, they can be active at temperatures below 10°C, in contrast with honeybees that are typically inactive when environmental temperature drops below 16°C (Dafni et al., 2010). *B. lucorum* (Fig. 2B), is more commonly found in the northern (border with Kosovo), and southern (Vlorë) areas of Albania (Goulson et al., 2008; Rasmont et al., 2008). It is widespread in temperate areas consisting of flowering herbaceous meadows, gardens, open forests and urban areas, where they forage for nectar and pollen (Tiranë, Berat, Lushnje Shkodër and Sarandë). Their nests can be very large containing up to 400 workers and are built underground, often in the abandoned nests of old mice (Rasmont et al., 2015). The honeybee, *A. mellifera*, has a wide distribution in various areas in the northern Albania (Kukës and Krujë), also in the western and south part of Albania (Durrës, Tiranë, Fier and Pogradec). The most common honeybee in Albania, *A. m. macedonica*, has morphological features that shows adaptations to the climatic conditions of the Balkan Peninsula from Eastern Albania to Bulgaria, allowing for a better tolerance of a range of temperatures (hot summers and cold winter) and different habitats such as mountains, valleys and areas with abundant wildflowers (Ruttner, 1988). Oriental hornets are large predatory hymenoptera that are colonized in new territories as a result of global warming, the globalized movement of people and goods. These remain as main factors that are facilitating the expansion of historical distribution of *V. orientalis*. The activity patterns of *V. orientalis* are different from the other hornets as these species are the only one able to use solar radiation to produce energy. Their cuticular pigments are able to absorb solar energy in the cuticle (Zucca et al., 2024). They are distributed in different parts of Albania, with more reported observation from iNaturalist in the south part areas as in Përmet, Tepelenë and Gjirokastër. The European social paper wasp *P. dominula,* is a present in the western areas of Albania in Durrës, Lushnje, Tiranë and Vlorë, inhabiting soft, terrestrial habitats such as forest and grassland biomes. These wasps can colonize nearby domestic environments due to abundant resources such as food and nesting materials (Weiner et al., 2012). The fire ant, *S. fugax, appears* to have a wide geographic range, spanning different regions where they prefer warm, dry, open areas with sparse vegetation, building their nests on the ground in rock crevices or under stones (Rigato and Toni, 2011). In Albania they have been reported from iNaturalist only in Krujë and Durrës. This species usually lives in the soil where it can infest crops and occurs in domestic environments due to the soil of indoor plants (Table 1) (Fig. 2B).

CONCLUSIONS

In Albania, some spiders and insects have been known to cause local or systemic symptoms in human victims following envenoming. However, only one spider can cause complications up to fatal cases, such as bites due to the neurotoxins of the venom of the black widow spider *L. tredecimguttatus*. In other species, severe cases may be associated with allergic reactions especially in relation to Hymenopterans. The main contributor to these serious allergic reactions may be the honeybee *A. mellifera*. Considering the importance of their bites and stings in Albania and further afield in the Balkans, and growing misconceptions about envenomings related by social and information media, we suggest that further research be carried to promote awareness and develop remediation plans.

ACKNOWLEDGEMENTS

We acknowledge the authors of the photos of spiders and insects for permitting to use them in this manuscript. The English language is improved using the academic program of Grammarly https://www.grammarly.com. We are thankful to Dr. Michel Dugon, National University of Galway, Ireland, for his valuable comments increasing the quality of this manuscript. National Agency for Scientific Research and Innovation (NASRI), Ministry of Education and Sport, Tirana, Albania, provided financial support for the project "*Venomous spiders of Albania: the black widow Latrodectus tredecimguttatus, distribution map and the importance of her venom in bitten case studies"*, on behalf of the National Program for Research and Development 2023 - 2024.

CONFLICT OF INTEREST STATEMENT.

The authors declare no conflict of interest.

REFERENCES

Adler, F. R., Green, A. M., and Şekercioğlu, Ç. H. (2020). Citizen science in ecology: a place for humans in nature. *Annals of the New York Academy of Sciences, 1469*(1), 52-64.

Ahrens, B., and Crocker, C. (2011). Bacterial etiology of necrotic arachnidism in black widow spider bites. *Journal of Clinical Toxicology, 1*(106), 2161-0495.1000106.

Anderson, B., Fagan, P., Woodnutt, T., and Chamorro-Premuzic, T. (2012). Facebook psychology: Popular questions answered by research. *Psychology of Popular Media Culture, 1*(1), 23.

Bagriacik, N. (2012). Comparison of the nest materials of *Polistes gallicus* (L.), *Polistes dominulus* (Christ) and *Polistes nimpha* (Christ)(Hymenoptera: Vespidae). *Archives of Biological Sciences, 64*(3), 1079-1084.

Bertsch, A., Schweer, H., and Titze, A. (2004). Discrimination of the bumblebee species *Bombus lucorum*, *Bombus cryptarum* and *Bombus magnus* by morphological characters and male labial gland secretions (Hymenoptera: Apidae). *Beiträge Zur Entomologie - Contributions to Entomology, 54*(2), 365-386.

Bettini, S., and Maroli, M. (1978). Venoms of theridiidae, genus *Latrodectus Arthropod venoms* (pp. 149-212): Springer.

Bilo, M., and Bonifazi, F. (2009). The natural history and epidemiology of insect venom allergy: clinical implications. *Clinical and Experimental Allergy, 39*(10), 1467-1476.

Bonney, R., Shirk, J. L., Phillips, T. B., Wiggins, A., Ballard, H. L., Miller-Rushing, A. J., and Parrish, J. K. (2014). Next steps for citizen science. *Science, 343*(6178), 1436-1437.

Brechman, J., Lee, C.-j., and Cappella, J. N. (2009). Lost in translation? A comparison of cancer-genetics reporting in the press release and its subsequent coverage in the press. *Science Communication, 30*(4), 453-474.

Bucher, C., Korner, P., and Wüthrich, B. (2001). Allergy to bumblebee venom. *Current opinion in allergy and clinical immunology, 1*(4), 361-365.

Burzyńska, M., and Piasecka-Kwiatkowska, D. (2021). A review of honeybee venom allergens and allergenicity. *International Journal of Molecular Sciences, 22*(16), 8371.

Canali, M., Hetoja, A., Peqini, I., and Segrè, A. (1998). Lessons from a diagnostic analysis of Albania's Divjaka region. *Land reform, land settlement and cooperatives [Reforme agraire, colonisation et cooperatives agricoles]. Reforma agraria, colonizacion y cooperativas, 1*, 137-150.

Caporiacco, L. d. (1932). Aracnidi raccolti in Albania dal dott. Pietro Parenzan. *Atti dell'Accademia VenetoTrentino-Istriana, 23*, 93-98.

Caporiacco, L. d. (1949). Alcuni aracnidi albanesi. *Atti del Museo Civico di Storia Naturale di Trieste, 17*(122-125).

Carvajal, M. A., Faúndez, E. I., and Pedemonte, G. G. (2023). On a bite case of *Steatoda triangulosa* (Walckenaer)(Arachnida: Theridiidae) on a human being. *Revista de la Sociedad Entomológica Argentina, 82*(1), 88-90.

Casewell, N. R., Wüster, W., Vonk, F. J., Harrison, R. A., and Fry, B. G. (2013). Complex cocktails: the evolutionary novelty of venoms. *Trends in ecology and evolution, 28*(4), 219-229.

Cetkovic, A. (2003). A review of the European distribution of the Oriental hornet (Hymenoptera, Vespidae: *Vespa orientalis* L.). *Ekologija, 37*(1), 22.

Chamberlain, J. (2018). Using social media for biomonitoring: how Facebook, Twitter, Flickr and other social networking platforms can provide large-scale biodiversity data *Advances in ecological research* (Vol. 59, pp. 133-168): Elsevier.

Cox, J., Oh, E. Y., Simmons, B., Lintott, C., Masters, K., Greenhill, A., Graham, G., and Holmes, K. (2015). Defining and measuring success in online citizen science: A case study of Zooniverse projects. *Computing in Science and Engineering*, *17*(4), 28-41.

Dafni, A., Kevan, P., Gross, C. L., and Goka, K. (2010). *Bombus terrestris*, pollinator, invasive and pest: An assessment of problems associated with its widespread introductions for commercial purposes. *Applied Entomology and Zoology*, *45*(1), 101-113.

Daly, N. L., and Wilson, D. (2018). Structural diversity of arthropod venom toxins. *Toxicon*, *152*, 46-56.

Dedej, S., Biasiolo, A., and Piva, R. (1996). Morphometric and alloenzymatic characterisation in the Albanian honeybee population *Apis mellifera* L. *Apidologie*, *27*(3), 121-131.

Deltshev, C., Vrenozi, B., Blagoev, G., and Lazarov, S. (2011). *Spiders of Albania– faunistic and zoogeographical review (Arachnida: Araneae)*.

Dugon, M. M., Dunbar, J. P., Afoullouss, S., Schulte, J., McEvoy, A., Hogan, R., Ennis, C., and Sulpice, R. (2017). *Occurrence, reproductive rate and identification of the non-native noble false widow spider Steatoda nobilis (Thorell, 1875) in Ireland.* Paper presented at the Biology and Environment: Proceedings of the Royal Irish Academy.

Dunbar, J. P., Afoullouss, S., Sulpice, R., and Dugon, M. M. (2018). Envenomation by the noble false widow spider *Steatoda nobilis* (Thorell, 1875)–five new cases of steatodism from Ireland and Great Britain. *Clinical Toxicology*, *56*(6), 433-435.

Dunbar, J. P., Fort, A., Redureau, D., Sulpice, R., Dugon, M. M., and Quinton, L. (2020). Venomics approach reveals a high proportion of *Lactrodectus*-like toxins in the venom of the noble false widow spider *Steatoda nobilis*. *Toxins*, *12*(6), 402.

Dunbar, J. P., Khan, N. A., Abberton, C. L., Brosnan, P., Murphy, J., Afoullouss, S., O’Flaherty, V., Dugon, M. M., and Boyd, A. (2020). Synanthropic spiders, including the global invasive noble false widow *Steatoda nobilis*, are reservoirs for medically important and antibiotic resistant bacteria. *Scientific Reports*, *10*(1), 20916.

Dunbar, J. P., Vitkauskaite, A., O’Keeffe, D. T., Fort, A., Sulpice, R., and Dugon, M. M. (2022). Bites by the noble false widow spider *Steatoda nobilis* can induce *Latrodectus*-like symptoms and vector-borne bacterial infections with implications for public health: a case series. *Clinical Toxicology*, *60*(1), 59-70.

Facebook community. Observations of *Latrodectus tredecimguttatus*, *Steatoda paykulliana*, *S. sp. nobilis*, *Apis mellifera*, *Bombus lucorum*, *B. pascuorum*, *B. terrestris*, *Polistes dominula*, *Vespa orientalis* from Albania, observed on/between 04.x.2013-24.v.2024. Exported from https://www.facebook.com on 10 October 2024.

Fitzgerald, K. T., and Flood, A. A. (2006). Hymenoptera stings. *Clinical techniques in small animal practice*, *21*(4), 194-204.

GBIF.org (2024), GBIF Home Page. Available from: https://www.gbif.org [10 October 2024].

GBIF.org (15 October 2024) GBIF Occurrence Download https://doi.org/10.15468/dl.4k8az4; https://doi.org/10.15468/dl.fvd6y9; https://doi.org/10.15468/dl.6cs3za

Gertsch, W. J., and Ennik, F. (1983). The spider genus *Loxosceles* in North America, Central America, and the West Indies (Araneae, Loxoscelidae). *Bulletin of the AMNH, 175*(3).

Golden, D. B. (2007). Insect sting anaphylaxis. *Immunology and allergy clinics of North America, 27*(2), 261-272.

Goulson, D., Lye, G. C., and Darvill, B. (2008). Decline and conservation of bumble bees. *Annu. Rev. Entomol., 53*, 191-208.

Graham, J. H., Hughie, H. H., Jones, S., Wrinn, K., Krzysik, A. J., Duda, J. J., Freeman, D. C.,

Emlen, J. M., Zak, J. C., and Kovacic, D. A. (2004). Habitat disturbance and the diversity and abundance of ants (Formicidae) in the Southeastern Fall-Line Sandhills. *Journal of Insect Science, 4*(1), 30.

Greenberg, L. (2009). Fire Ants *Encyclopedia of Insects* (pp. 362-364): Elsevier.

Gregori, J., and Starman, B. (2003). *The carniolan bee (Apis mellifera carnica) in Slovenia*: Beekeepers' Association of Slovenia.

Grosch, J., Eberlein, B., Waldherr, S., Pascal, M., San Bartolomé, C., De La Roca Pinzón, F., Dittmar, M., Hilger, Ch., Ollert, M., and Biedermann, T. (2021). Characterization of new allergens from the venom of the European paper wasp *Polistes dominula*. *Toxins, 13*(8), 559.

Grosch, J., Hilger, C., Bilo, M. B., Kler, S., Schiener, M., Dittmar, G., Bernardin, F., Lesur, A., Ollert, M., and Schmidt-Weber, C. B. (2020). Shedding Light on the Venom Proteomes of the Allergy-Relevant Hymenoptera *Polistes dotninula* (European Paper Wasp) and *Vespula* spp.(Yellow Jacket). *Toxins, 10*(5), 1av+.

Hoffman, D. R., and Jacobson, R. S. (1996). Allergens in Hymenoptera venom XXVII: Bumblebee venom allergy and allergens. *Journal of allergy and clinical immunology, 97*(3), 812-821.

Hörweg, C. (2023). Der Ammendornfinger *Cheiracanthium punctorium.* Spinne des Jahres 2023–berühmt-berüchtigt unter den Spinnen. *Entomologica Austriaca.*(30), 297-302.

iNaturalist. Available from https://www.inaturalist.org. Retrieved Accessed [10 October 2024]

iNaturalist community. Observations of *Latrodectus tredecimguttatus, Steatoda paykulliana, S. triangulosa, Apis mellifera, Bombus lucorum, B. pascuorum, B. terrestris, Polistes dominula, Solenopsis fugax, Vespa orientalis* from Albania, observed on/between 08.ii.2017-28.v.2024. Exported from https://www.inaturalist.org on 10 October 2024.

Infantidis, M. (1979). Morphological characters of the Greek bee *Apis mellifica cecropia*. *Apiacta, 14*(3), 109-114.

Keyler, D., Ahmad, M., Rodriguez, A., and De Silva, P. (2020). *Latrodectus geometricus* (Aranea: Theridiidae) envenoming: Rapid resolution of symptoms following F (ab') 2 antivenom therapy. *Toxicon, 188*, 76-79.

King, T. P., and Spangfort, M. D. (2000). Structure and biology of stinging insect venom allergens. *International archives of allergy and immunology, 123*(2), 99-106.

Kittelberger, K. D., Hendrix, S. V., and Şekercioğlu, Ç. H. (2021). The value of citizen science in increasing our knowledge of under-sampled biodiversity: An overview of public documentation of Auchenorrhyncha and the Hoppers of North Carolina. *Frontiers in Environmental Science, 9*, 710396.

Korman, S., Jabbour, S., and Harari, M. (1990). Multiple hornet (*Vespa orientalis*) stings with fatal outcome in a child. *Journal of paediatrics and child health, 26*(5), 283-285.

Kovács, G., and Szinetár, C. (2018). [Data on the biology of the cupboard spider: *Steatoda grossa* (C. L. Koch, 1838), (Araneae, Theridiidae)]. *Biológia Savaria Természettudományi és Sporttudományi Közlemények* (17), 91-108.

Kuliçi, M., Kokthi, E., Hasani, A., Zoto, O., Marcelino, J. A., and Giordano, R. (2023). Threats and drivers of change in populations of managed honey bees (*Apis mellifera*) in the Balkan countries of Albania and Kosovo. *Bulletin of Insectology, 76*.

Kůrka, A., Naumova, M., Indzhov, S., and Deltshev, C. (2020). New faunistic and taxonomic data on the spider fauna of Albania (Arachnida: Araneae). *Arachnologische Mitteilungen: Arachnology Letters*, *59*(1), 8-21.

Lecocq, T., Brasero, N., Martinet, B., Valterova, I., and Rasmont, P. (2015). Highly polytypic taxon complex: interspecific and intraspecific integrative taxonomic assessment of the widespread pollinator *Bombus pascuorum* Scopoli 1763 (Hymenoptera: Apidae). *Systematic Entomology, 40*(4), 881-890.

Levy, G., and Amitai, P. (1983). Revision of the widow-spider genus *Latrodectus* (Araneae: Theridiidae) in Israel. *Zoological Journal of the Linnean Society*, *77*(1), 39-63.

Lister, M. (2017). Essential Social Media Marketing Statistics For 2017," Wordstream: Wordstream. com. Retrieved August 30th.

Mammola, S., Nanni, V., Pantini, P., and Isaia, M. (2020). Media framing of spiders may exacerbate arachnophobic sentiments. *People and Nature*, *2*(4), 1145-1157.

Maretić, Z., Levi, H. W., and Levi, L. R. (1964). The theridiid spider *Steatoda paykulliana*, poisonous to mammals. *Toxicon, 2*(3), 149-154.

Mingomataj, E., Ohri, D., Dhimitri, V., Priftanji, A., Qirko, E., Pani, L., Fischer, T. C., Dinh, Q. T., Peiser, C. and Fischer, A. (2003). Hymenoptera sting anaphylactic reactions in the Mediterranean population of Albania. *Journal of Investigational Allergology and Clinical Immunology*, *13*(4), 272-277.

Mingomataj, E., Priftanji, A., Qirko, E., Dinh, Q. T., Fischer, A., Peiser, C., and Groneberg, D. A. (2002). Specific immunotherapy in Albanian patients with anaphylaxis to hymenoptera venoms. *BMC dermatology, 2*, 1-6.

Mukund, H., and Gawade, S. (2011). Evaluation of effects of photooxidized *Vespa orientalis* venom on memory and learning in rats. *Journal of Venomous Animals and Toxins including Tropical Diseases, 17*, 422-429.

Nakajima, T., Yasuhara, T., Uzu, S., Wakamatsu, K., Miyazawa, T., Fukuda, K., and Tsukamoto, Y. (1985). Wasp venom peptides; wasp kinins, new cytotrophic peptide families and their physico-chemical properties. *Peptides*, *6*, 425-430.

Naumova, M. (2020). Descriptions of two new spider species, with new data on the Albanian arachnofauna (Arachnida: Araneae, Opiliones, Pseudoscorpiones and Scorpiones). *Acta zoologica bulgarica*, *72*(1), 3-12.

Naumova, M., and Deltshev, C. (2021). New faunistic and taxonomic notes on the haplogyne and cribellate spiders (Araneae: Dictynidae, Dysderidae, Eresidae, Filistatidae, Sicariidae) from three Balkan countries. *Acta Zoologica Academiae Scientiarum Hungaricae, 67*(1), 63-76.

Nelkin, D. (1995). Selling Science. How the Press Covers Science and Technology. *New York: W. H. Freeman and Company*.

Nelsen, D. R., Kelln, W., and Hayes, W. K. (2014). Poke but don't pinch: risk assessment and venom metering in the western black widow spider, *Latrodectus hesperus*. *Animal Behaviour, 89*, 107-114.

Nentwig, W., Blick, T., Bosmans, R., Gloor, D., Hänggi, A., and Kropf, C. (2024). Spiders of Europe. Version 01.2024. Retrieved 10 October 2024, from https://www.araneae.nmbe.ch

Paolino, G., Di Nicola, M., Di Pompeo, P., Dorne, J., and Mercuri, S. (2021). Key to medically relevant Italian spider bites: a practical quick recognition tool for clinicians. *La Clinica Terapeutica, 172*(4).

Papini, R. (2012). Documented bites by a yellow sac spider (*Cheiracanthium punctorium*) in Italy: a case report. *Journal of Venomous Animals and Toxins including Tropical Diseases, 18,* 349-354.

Peterson, M. E. (2006). Black widow spider envenomation. *Clinical techniques in small animal practice, 21*(4), 187-190.

Plowright, C., Plowright, R., and Williams, P. H. (1997). Replacement of *Bombus muscorum* by *Bombus pascuorum* in Northern Britain. *The Canadian Entomologist, 129*(6), 985-990.

Pradervand, J.-N., Pellissier, L., Rossier, L., Dubuis, A., Guisan, A., and Cherix, D. (2011). Diversity of bumblebees (*Bombus* Latreille, Apidae) in the Alps of the canton Vaud (Switzerland).

Rasmont, P., Coppée, A., Michez, D., and De Meulemeester, T. (2008). *An overview of the Bombus terrestris (L. 1758) subspecies (Hymenoptera: Apidae).* Paper presented at the Annales de la Société entomologique de France.

Rasmont, P., Franzen, M., Lecocq, T., Harpke, A., Roberts, S. P., Biesmeijer, J. C., Castro, L., Cederberg, B., Dvorak, L., and Fitzpatrick, U. (2015). *Climatic risk and distribution atlas of European bumblebees* (10): Pensoft Publishers.

Rigato, F., and Toni, I. (2011). Short notes 21. Hymenoptera, Formicidae. *Research in the framework of the ICP Forests network. Conservazione Habitat Invertebrati, 5,* 873-882.

Rohou, A., Nield, J., and Ushkaryov, Y. (2007). Insecticidal toxins from black widow spider venom. *Toxicon, 49*(4), 531-549.

Romita, P., Foti, C., Principato, M., and Stingeni, L. (2018). Generalized papular-purpuric eruption due to *Solenopsis fugax* bites. *Anais brasileiros de dermatologia, 93*(4), 570-572.

Ruttner, F. (1988). Biogeography and taxonomy of honeybees Springer. *Berlin, Germany*.

Saez, N. J., Senff, S., Jensen, J. E., Er, S. Y., Herzig, V., Rash, L. D., and King, G. F. (2010). Spider-venom peptides as therapeutics. *Toxins, 2*(12), 2851-2871.

Schmidt, J. O. (2019). Arthropod toxins and venoms *Medical and veterinary entomology* (pp. 23-31): Elsevier.

Schmidt, S., and Smith, D. (2006). An annotated systematic world catalogue of the Pergidae (Hymenoptera).

Seltzer, C. (2019). Making Biodiversity Data Social, Shareable, and Scalable: Reflections on iNaturalist and citizen science. *Biodiversity Information Science and Standards*(e10197).

Shafaie, S., Dabiri, F., Badakhshan, M., Naghian, A., Rafinejad, J., Sh, S., and Rasegh, P. (2021). New data *on Latrodectus tredecimguttatus* Rossi, 1790, the medically important spider species (Araneae: Theridiidae) from Iran. *Archives of Razi Institute, 76*(2), 385.

Shulov, A., and Weissman, A. (1959). Notes on the life habits and potency of the venom of the three *Latrodectus* spider species of Israel. *Ecology, 40*(3), 515-518.

Stingeni, L., Mariotti, M., and Principato, M. (2002). Papular urticaria from *Solenopsis fugax* (Hymenoptera: Formicidae) in domestic environments. *Annali Italiani di Dermatologia Allergologica Clinica e Sperimentale, 56*(1), 21-23.

Strand, E. (1919). Spinnen und Opiliones aus Griechenland, Albanien und Kleinasien. *Archiv für Naturgeschichte, 82*(A2), 158-167

Thill, V. L., Moniz, H. A., Teglas, M. B., Wasley, M. J., and Feldman, C. R. (2022). Preying dangerously: black widow spider venom resistance in sympatric lizards. *Royal Society Open Science, 9*(10), 221012.

Tibbetts, E. A., Skaldina, O., Zhao, V., Toth, A. L., Skaldin, M., Beani, L., and Dale, J. (2011). Geographic variation in the status signals of *Polistes dominulus* paper wasps. *PloS one, 6*(12), e28173.

van Helsdingen, P. J., and IJland, S. (2015). A quick scan of the spider fauna in the surroundings of Përmet, Albania (Arachnida, Araneae)–Preliminary report. *Nieuwsbrief SPINED, 35*, 15-33.

Velthuis, H. H., and Van Doorn, A. (2006). A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie, 37*(4), 421-451.

Vitkauskaite, A., Dunbar, J. P., Lawton, C., Dalagiorgos, P., Allen, M. M., and Dugon, M. M. (2021). Vertebrate prey capture by *Latrodectus mactans* (Walckenaer, 1805) and *Steatoda triangulosa* (Walckenaer, 1802)(Araneae, Theridiidae) provide further insights into the immobilization and hoisting mechanisms of large prey. *Food Webs, 29*, e00210.

Vrenozi, B. (2012). A collection of spiders (Araneae) in Albanian coastal areas. *Arachnologische Mitteilungen, 44*, 41-46.

Vrenozi, B. (2022). Venomous spiders of Albania–does an increase of temperature influence the toxicity of spider venom? *Toxicon: X, 15*, 100135.

Vrenozi, B., and Dunlop, J. A. (2013). Albanian arachnids in the Museum für Naturkunde, Berlin. *Arachnology, 16*(1), 10-15.

Vrenozi, B., and Haxhiu, I. (2008). *Data on order Araneae (Class Arachnida) in the Western Adriatic Lowland.* Paper presented at the Proceedings of International Conference on Biological and Environmental Sciences.

Vrenozi, B., and Jäger, P. (2013). Spiders (Araneae) from Albania and Kosovo in the collection of Carl Friedrich. *Arachnologische Mitteilungen, 46*, 17-26.

Wagner, H. C., Seifert, B., Borovsky, R., and Paill, W. (2018). First insight into the ant diversity of the Vjosa valley, Albania (Hymenoptera: Formicidae). *Acta ZooBot Austria, 155*, 315-321.

Walker, A. A., Robinson, S. D., Yeates, D. K., Jin, J., Baumann, K., Dobson, J., Fry, B. G., and King, G. F. (2018). Entomo-venomics: The evolution, biology and biochemistry of insect venoms. *Toxicon, 154*, 15-27.

Warrell, D., Shaheen, J., Hillyard, P., and Jones, D. (1991). Neurotoxic envenoming by an immigrant spider (*Steatoda nobilis*) in southern England. *Toxicon, 29*(10), 1263-1265.

Weiner, S., Noble, K., Upton, C., Flynn, G., Woods, W., and Starks, P. (2012). The cost of flight: a role in the Polistes dominulus invasion. *Insectes sociaux, 59*, 81-86.

Weinstein, S. A. (2024). Hypersensitivity vs. uncommonly severe local envenoming by the red-back spider, *Latrodectus hasselti* Thorell, 1870 (Araneae: Theridiidae). *Toxicon, 249*, 108077.

Wigger, E., Kuhn-Nentwig, L., and Nentwig, W. (2002). The venom optimisation hypothesis: a spider injects large venom quantities only into difficult prey types. *Toxicon, 40*(6), 749-752.

World Spider Catalog. (2024). World Spider Catalog. Version 24.5. Retrieved 04 October 2024, from http://wsc.nmbe.ch

Zucca, P., Granato, A., Mutinelli, F., Schiavon, E., Bordin, F., Dimech, M., Balbo, R. A., Mifsud, D., Dondi, M., and Cipolat-Gotet, C. (2024). The oriental hornet (*Vespa orientalis*) as a potential vector of honey bee's pathogens and a threat for public health in North-East Italy. *Veterinary Medicine and Science, 10*(1), e1310.

.**Table 1**. The data on the distribution of venomous spiders and insects in Albania based on the citizen science, mass media and research papers

Class	Species	Location	Habitat	Date	Information	Author
Arachnida	*Cheiracanthium mildei*	Berat (Bogovë, Osumi river, Velabisht)	Different vegetation	04.vii.2012; 01.vi.2023	Observed	Kurka et. al. (2020); GBIF.org
		Korcë (Leskovik, Kolonjë)	Along small rivulet, grassland, shrubs, trees	30.iv.2014	Observed	van Helsdingen and Ijland (2015)
		Sarandë (Lukovë)	Different vegetation	05.vi.1995	Observed	Deltshev et. al. (2011)
		Vlorë (Himarë)	Rocky slopes	06.vi.2013	Observed	Kurka et. al. (2020)
	Cheiracanthum puntorium	Tiranë	Botanical Garden	07.vi.2007	Observed	Vrenozi and Haxhiu (2008); Vrenozi (2012)
		Vlorë (Nartë)	Sandy dunes	vii.2018	Observed	B. Vrenozi pers.com
	Latrodectus tredecimguttatus	Berat	Agriculture	20.vi.2023	Spider bites	Shqiptarja.com
		Elbasan (Dumre)	Agriculture	26-28.vi.2024	Spider bites	InFormim.al, Infoweb
		Fier (Seman, Mallakastër, Rroskovec)	Agriculture	09.vii.2021; 28.vi.2023	Spider bites (severe chest pain)	Syri TV; Balkan web
		Konispol (Vrinë)	Dried vegetation	05.vi.2013	Observed	Kurka et. al. (2020)
		Korcë (Leskovik)	Dried vegetation	10.vi.2012	Observed	Kurka et. al. (2020)
		Lushnjë (Bedat, Divjakë, Grabian, Karavasta, Tërbuf)	Agriculture, walls of the buildings, dried land of the Karavasta lagoon, villages around the Karavasta lagoon	vii.2019; 18.vi.2029' 25.vi.2020; 16.x.2021; 09.vii.2022; 29.vi./04-21.vii.2023;	Spider bites (severe pain, two deaths), observed female (black, black with orange patterns, black with red patterns)	lajme.rtsh.al, Lushnja.info, Facebook community, iNaturalist community, Vrenozi (2022)
		Rrogozhinë	Agriculture	27.vi.2022	Spider bites (life threatening)	RTK 4+

Class	Species	Location	Habitat	Date	Information	Author
		Tepelenë (Memaliaj)	in the stony walls	04.x.2013; 28.i.2014; 27.vi.2023	Observed female (black with orange, and red patterns)	Facebook community, iNaturalist community
	Loxosceles rufescens	Fier	Inside home in the city	21.vii.2021	Spider bite (necrosis)	Vrenozi (2022)
		Sarandë	Open, dry, and sunny habitat without vegetation, between newly built houses, under stones.	10.vi.2019	Observed	Naumova and Deltshev (2021)
		Tiranë	In the walls in the city	vii.2014	Observed	Vrenozi (2022)
Arachnida	*Steatoda grossa*	Durrës	NA	25.ix.2021; 05.viii.2023	Observed	GBIF.org
	Steatoda sp. nobilis	Tiranë (Fresk, Kombinat)	Inside home bitten while wearing pants in the morning	04.vii.2022	Spider bites (infection, surgical intervention, necrosis)	B. Vrenozi pers.com
		Vlorë (Kaninë)	Under dried vegetation and stones	08.ii.2017	Observed	Facebook community
	Steatoda paykulliana	Dibër	Korab Mt.	1935	Observed	Vrenozi and Jaeger (2013)
		Durrës	Two km north from Durrës, Robi Mt., along the coast, stony area	26.v.1993; 03.vi.2008; 11.v.2024	Observed	Deltshev et al. (2011); Vrenozi (2012); Caporiacco (1932); GBIF.org
		Përmet (Alipostivan, Bënjë)	Grassy area with shrubs; slope with stones on soil; along Vjosa river	22.iv.2014; 03.v.2014; 20.v.2018; 17.vi.2019	Observed	van Helsdingen and Ijland (2015); B. Vrenozi leg.; GBIF.org
		Pukë (Lajthizë)	Different vegetation	2.vi.2014	Observed	Kurka et. al. (2020)
		Sarandë (Lukovë)	Ionian coast	05.vi.1995; 04.v.2024	Observed	Deltshev et al. (2011); iNaturalist community
		Shkodër (Velipojë)	Different vegetation	04.v.2023	Observed	GBIF.org

Class	Species	Location	Habitat	Date	Information	Author
		Tiranë	Dajti Mt.; Vorë hill	04.vi.2008; 15.iv.2008	Observed	Vrenozi and Haxhiu (2008); Vrenozi (2012)
		Vlorë (Dukat,Kaninë; Llogara, Orikum)	Different vegetation	1887; v.1931; 10.v.1995; 08.ii.2017; 16.v.2017; 17.xi.2023; 21-22.iv.2024	Observed	Strand (1919); Caporiacco (1949); Deltshev et al. (2011); Vrenozi and Dunlop (2013); GBIF.org; iNaturalist community; Facebook community
		Durrës	Different vegetation; around the buildings	29.vii.2019; 26.v.1993; 18.vii.2021; 07.09.2022; 20.v.2023; 29.x.2023	Observed	Deltshev et. al. (2011); GBIF.org
		Fier (Apolloni)	Around the buildings	27.iv.2008	Observed	Vrenozi and Haxhiu (2008); Vrenozi (2012)
	Steatoda triangulosa	Gjirokastër	NA	16.vi.2023	Observed	GBIF.org
	Steatoda triangulosa	Përmet (city, Petran, Strëmbec)	Around the hotel in the city; artificial Gallery; stony area	07.v.1995; 22-27.iv.2014; 17.vi.2019	Observed	Deltshev et al. (2011); van Helsdingen and Ijland (2015); GBIF.org
		Sarandë (Ksamil, Jermë, Shalës)	Different vegetation; around the buildings	28.ix-04.x.2018; 28.vi.2021	Observed	Naumova (2020); iNaturalist community
	Steatoda triangulosa	Vlorë (Himarë, Jalë)	Different vegetation, stony area	28.ix-04.x.2018; 14.vii.2017; 02.viii.2017	Observed	Naumova (2020); iNaturalist community
Insecta	*Apis mellifera*	Berat	Resting on a flower	6.xi.2022	Observed (dark body with brown belly stripes)	iNaturalist community
		Burrel	At the hive	iv-v.1993	Observed	Dedej et. al. (1996)
		Durrës	On the wire, on the ground	01.i.2024; 22.iii.2023	Observed	GBIF.org
		Elbasan	NA	14.viii.2023	Insect bite	a2news

Class	Species	Location	Habitat	Date	Information	Author
		Fier	Resting on a flower	14.iii.2018	Observed	iNaturalist community
		Kavajë	At the hive	iv-v.1993	Observed	Dedej et. al. (1996)
		Krujë	At the hive	iv-v.1993	Observed	Dedej et. al. (1996)
		Kukës	At the hive	iv-v.1993	Observed	Dedej et. al. (1996)
		Lezhë (Kune Vain)	Resting on a flower	6.viii.2022	Observed	iNaturalist community
		Lushnje	Resting on a flower	31.iii.2018; 24.v.2024	Observed	Facebook community; iNaturalist community
		Pogradec	At the hive	iv-v.1993	Observed	Dedej et. al. (1996)
		Sarandë	At the hive	iv-v.1993	Observed	Dedej et. al. (1996)
		Tepelenë	At the hive	iv-v.1993	Observed	Dedej et. al. (1996)
		Tiranë	Different vegetations, city, resting on a flower	1987-1996; 20.iv.2024 12.i.2023	Insect bite (anaphylactic reactions); observed	Dedej et. al. (1996); Mingomataj et al. (2002, 2003); GBIF.org
		Vlorë	Resting on a flower	13.iii.2017 23.iv.2024	Observed	iNaturalist community, GBIF.org
	Bombus lucorum	Berat (Tomorri Mt.)	Resting on a flower	27.v.2024	Observed (lemon yellow and dark body with white tail)	iNaturalist community
		Lushnje (Divjakë)	Resting on a flower	11.xi.2022	Observed	iNaturalist community
		Sarandë (Delvinë)	Resting on a flower	06.v.2017;	Observed	iNaturalist community
		Shkodër (Valbonë)	Resting on a flower	09.viii.2022	Observed	iNaturalist community
		Tiranë	Resting on a flower	13.viii.2023; 20.iii.2024	Observed	iNaturalist community
Insecta		Vlorë (Sazani island, Karaburun Peninsula, city)	Resting on a flower, different vegetations	22.xi.2016; 05.iv.-22.vii.2019; ii.2020; 23.iii.2024	Observed	Facebook community, iNaturalist community
	B. pascuorum	Kukës	Resting on a flower	09.viii.2022	Observed (Orange with black and white body)	iNaturalist community
		Shkodër (Theth)	Resting on a flower	28.v.2024	Observed	iNaturalist community

Class	Species	Location	Habitat	Date	Information	Author
		Vlorë (Llogara)	Resting on a flower	21.iv.2019	Observed	iNaturalist community
		Tiranë (Dajti Mt., Pezë)	Resting on a flower	05.04.2023; 13.viii.2023	Observed	Facebook community, iNaturalist community
	B. terrestris	Lushnje (Divjakë)	Resting on a flower	04.xi.2022	Observed (ginger color with white tail)	iNaturalist community
		Durrës	Resting on a flower	18.x.2019	Observed	iNaturalist community
		Has	Resting on a flower	19.v.2024	Observed	Facebook community
		Vlorë (Llogara)	Resting on a flower	02.iii.2020	Observed	Facebook community
	Polistes dominula	Dibër (Mat)	On the leaf	26.v.2024	Observed (Yellow and dark body)	iNaturalist community
		Durrës (Arapaj, Beach, Lalzi Bay, Sektori Rinia, Spitallë)	Low and high vegetation, beach area, on their nests	24.x.2016; 01.i.-27.viii.2019; 14.iv-30.v.2020; 25.ii./21.iv./05-19.ix.2021; 20.iv.2024	Observed	Facebook community, iNaturalist community
		Lushnjë (Divjakë)	Resting on a flower	30.x.2020	Observed	iNaturalist community
		Korcë (Kolonjë)	Resting on a flower	23.vi.2023	Observed	iNaturalist community
		Shkodër	On the ground	06.viii.2022	Observed	iNaturalist community
		Tiranë (Kamëz)	Low vegetation, on their nest	22.v.2020; 07.v.2021	Observed	Facebook community
		Vlorë (Zvërnec)	Resting on a flower	21.ix.2021	Observed	Facebook community
	Solenopsis fugax	Durrës	On the ground	29.x. 2023	Observed (brown body)	iNaturalist community
		Krujë	In the hand of the photographer	30.ix.2022	Observed	iNaturalist community
	Vespa orientalis	Durrës	On the ground	15.x.2019	Observed (yellow and dark body)	iNaturalist community
		Elbasan	In the clothes hanger; on the ground	2003; 11.viii.2023	Observed; Insect bite	Cetkovic (2003); iNaturalist community; a2news
	Vespa orientalis	Fier	In stone	23.x.2018	Observed	iNaturalist community

Clas s	Species	Location	Habitat	Date	Informatio n	Author
		Gjirokastër	On the rock	16.vi.2022	Observed	iNaturalist community
		Himarë (Porto Palermo)	On the ground	14.ix.2020	Observed	Facebook community
		Përmet	In stone	27.viii.2022	Observed	iNaturalist community
		Tepelenë (Memaliaj)	In stone	27.vi.2023	Observed	iNaturalist community
		Tiranë	On the pole	3.xi.2023	Observed	iNaturalist community
		Vlorë (Kaninë)	On the ground, in water pipes	22.ix.2019; 10.vi.2020; 09.vi.2020	Observed	Facebook community; iNaturalist community

EFFECT OF COTYLEDON LEAF DAMAGE ON SEEDLING GROWTH IN COTTON

Mehmet Demir KAYA[1], Engin Gökhan KULAN[1], Nurgül ERGİN[2], Pınar HARMANCI[1], Elif YAMAN[1]*

[1] Eskişehir Osmangazi University, Faculty of Agriculture, Department of Field Crops, Eskişehir, Türkiye
[2] Bilecik Şeyh Edebali University, Faculty of Agriculture and Natural Sciences, Department of Field Crops, Bilecik, Türkiye

Corresponding author e-mail: nurgulergin180@gmail.com

ABSTRACT

Cotyledons are the first photosynthetic organs of plants and play an important role in seedling growth. However, various biotic and abiotic factors can damage cotyledonary leaves shortly after seedling emergence. In this study conducted to determine the effect of cotyledon damage on seedling development in cotton plants, one (50% damage) and two (100% damage) of the cotyledons were removed from 15-day-old seedlings of cotton grown under laboratory conditions. No cotyledonary leaf damage was applied to the control group. Three weeks after the application, shoot length, stem diameter, leaf number, root length, shoot fresh and dry weight, and root fresh and dry weight were measured. The results showed that seedlings with a single cotyledon had higher shoot length by 0.9 cm, root fresh and dry weight by 22 mg plant^{-1} and 67 mg plant^{-1}, respectively. Compared to control plants, root length decreased by 1 cm, stem diameter by 0.3 mm and shoot fresh and dry weight by 260 mg plant^{-1} and 100 mg plant^{-1}, respectively. There was a significant decrease in other characteristics except leaf number in seedlings with both cotyledons removed. It was concluded that the cotton plants could tolerate moderately damaged cotyledon leaves, but development was significantly retarded in plants without cotyledons.

Keywords: *Gossypium hirsutum* L., cotyledon leaf damage, seedling growth

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important industrial plants that provides raw materials for many industrial sectors, such as textile, oil, and feed. Its high adaptability to different environmental conditions allows cotton to grow even in regions with arid and semi-arid climates with irrigation (Ödemiş et al., 2018). Approximately 777 thousand tons of fiber were obtained from 2.1 million tons of seed produced on an area of 470 thousand hectares. Also, 170 thousand tons of cooking oil and 793 thousand tons of oil cake were produced in 2023. Globally, Türkiye ranks sixth in cotton cultivation area, ninth in cotton output volume, third in cotton consumption, and fourth in cotton imports (Anonymous, 2024; TUİK, 2024).

Cotton plants can be damaged by biotic and abiotic factors throughout plant development, especially in the early seedling stage. While herbivorous pests cause physical damage the leaves, stem, flowers and fruits of cotton, abiotic factors such as drought, high temperatures and excessive rainfall cause deterioration in leaf tissue. Damage or loss of cotyledonary leaves significantly impacts plant development and yield (Green and Minton, 1980). Because cotyledons contain both the nutrients needed by the plant in the first development stage and contribute to nutrient accumulation by photosynthesis from the moment, they first emerge from the soil surface. In this way, root, shoot, and leaf growth continue depending on cotyledon leaves (Bisognin et al., 2005).

Damage to cotyledon leaves negatively affects seedling development. It was determined in several studies conducted on 17 plant species by Hanley and May (2006), Iortsuun et al. (2008), Moscardi et al. (2012), Hu et al. (2016) and Chen et al. (2023) that cotyledon loss negatively affects plant height, stem diameter, seedling fresh and dry weight. The aim of this study was to investigate the impact of damage level on cotyledon leaves on seedling development in cotton plants.

MATERIAL AND METHOD

This research was conducted in 2022 at the Seed Science and Technology Laboratory, Department of Field Crops, Faculty of Agriculture, Eskişehir Osmangazi University. The BA 1010 cotton variety from ProGen Seed was used as a plant material. The seeds were sowed in the pots with 9 cm diameter and 14 cm height, containing a mixture of field soil: perlite: vermiculite (6:1:1) to a depth of 3 cm. The pots were kept in a plant growth chamber set at 25℃/15℃ (16/8 hours day/night). In addition, all pots were watered with 10 mL of tap water every day until the research was completed, and 0.5 g of ammonium nitrate fertilizer dissolved in water was given to each pot three times.

On the 15th day of the study (when the first true leaf is just beginning to appear), 3 groups were designed with 4 pots in each group to create cotyledon leaf damage in emerging plants (Figure 1).

Group 1 (Control- 0% damage): Cotyledon leaves were left on the plant,

Group 2 (50% damage): One of the cotyledon leaves was removed from the plant,

Group 3 (100% damage): Both cotyledon leaves were removed from the plant.

On the 40th day after planting, the plants were harvested and shoot length, root length, stem diameter and leaf number, shoot fresh and root fresh weight values were measured. After the seedlings were kept at 80℃ for 24 hours, shoot dry and root dry weight values were weight.

Figure 1. Different cotyledonary leaf damages in cotton plants.

At the end of the research, One-Way ANOVA was performed in accordance with the completely randomized design (CRD) and the differences between the means were determined with the LSD ($p < 0.05$) test using the MSTAT-C (Michigan State University,

v. 2.10) program.

RESULTS AND DISCUSSION

The results showed that cotyledonary leaf damage significantly affected seedling growth of cotton. The variance analysis results of shoot and root length, stem diameter, and leaf number are given in Table 1.

Table 1. Variance analysis of the traits examined in cotton with cotyledon damage

Source of variation	DF	Mean squares			
		Shoot length	Root length	Stem diameter	Leaf number
Leaf damage	2	6.528**	10.901**	0.211**	0.250
Error	9	0.099	0.706	0.012	0.167
Total	11				

**: Significant at $p < 0.01$.

Shoot length, root length, stem diameter, and leaf number in cotton were significantly influenced by different cotyledon damages, and significant differences were detected at $p < 0.01$ (Table 1). The mean values and differences of these examined traits are summarized in Table 2.

Table 2. Changes in shoot and root length, stem diameter and leaf number of cotton seedling under cotyledon damage.

	Shoot length (cm)	Root length (cm)	Stem diameter (mm)	Leaf number (number)
Control	6.50^{b}	15.2^{a}	2.21^{a}†	3.3
50% damage	7.43^{a}	14.2^{a}	1.93^{b}	3.0
100% damage	4.90^{c}	12.0^{b}	1.76^{b}	2.8

†: Different letters indicate significance levels at $p < 0.05$.

Damage to cotyledons significantly affected both shoot and root growth in cotton. Especially, the removal of both cotyledonary leaves significantly reduced the shoot and root lengths of seedlings (Table 2). Cotyledons are the first photosynthetic organs of the plant, which are necessary for the initial growth stage and dry matter accumulation in plants. Many studies have suggested that even if cotyledons are damaged due to different biotic or abiotic sources, the remaining cotyledons can still support seed germination and contain sufficient nutrients to support early growth of seedlings (Xiao et al., 2013; Chen et al., 2023). In addition, shoot and root growth are thought to have different developmental responses in cotyledon damage. Bisognin et al. (2005) reported that shoot growth in cucumber seedlings was less dependent on cotyledon leaves than root growth. Since root growth occurs using nutrients produced by cotyledons, it was determined that plants with completely removed cotyledons (1.76 cm) had shorter roots than the control (2.21 cm) (Table 2). Similar results were obtained in the number of leaves and stem diameter as well as the decrease in plant height. Chen et al. (2023) also found that the leaf growth and stem diameter of *Quercus acutissima* decreased significantly in cases of cotyledon damage.

Table 3. Variance analysis of the traits examined in cotton with cotyledon damage

Source of variation	DF	Mean squares			
		Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight
Leaf damage	2	1.737**	0.191**	65815**	12482**
Error	9	0.004	0.005	222	105
Total	11				

**: Significant at $p < 0.01$.

The variance analysis results for shoot and root fresh weight and shoot and root dry weight are given in Table 3. All the traits examined were found to be statistically significant at $p < 0.01$. The mean values and significance levels of these traits are given in Table 4.

Table 4. Changes in shoot and root fresh weight and shoot and root dry weight of cotton seedling under cotyledon damage.

	Shoot fresh weight (g plant^{-1})	Root fresh weight (g plant^{-1})	Shoot dry weight (mg plant^{-1})	Root dry weight (mg plant^{-1})
Control	2.12^{a}	0.90^{a}	363^{a}	136^{b}†
50% damage	1.86^{b}	0.92^{a}	263^{b}	203^{a}
100% damage	0.87^{c}	0.53^{b}	108^{c}	92^{c}

†: Different letters indicate significance levels at $p < 0.05$.

The fresh and dry weights of shoot and root were significantly depressed by cotyledon damages. The highest shoot fresh weight (2.12 g plant^{-1}) was determined in control plants, while the lowest shoots (0.87 g plant^{-1}) were obtained from plants treated with 100% cotyledon damage (Table 4). Similar results were determined in root fresh weight. The results of Kaya and Bayramin (2013) support our findings, who found a significant reduction in plant fresh weight of sunflower.

Since cotyledons are both seed storage organs and photosynthetic organs, they are of great importance in meeting the energy and nutrient needs of young seedlings. Therefore, cotyledon damage that may occur at the early seedling stage negatively affects plant growth and development (Wallace and Eigenbrode, 2002; Kitajima, 2003). Damage to all or part of the cotyledons means that a large part of the storage reserves is also damaged, especially for seedlings that have not reached sufficient photosynthetic area to compensate for this damage (Kennedy et al., 2004; Hanley and Fegan, 2007). In this study, a 27% decrease in shoot fresh weight occurred in the case of removal of a single cotyledon, this rate was 70% in plants where both cotyledons were removed. The lowest dry weight (92 mg plant^{-1}) was determined in cotton plants damaged by 100% cotyledonary leaf removal (Table 4).

CONCLUSION

Cotyledonary leaf damage may have hazardous effects on seedling growth and later stages of plant development. In this study, the effects of different levels of cotyledon damage on early seedling development in cotton were investigated. It was concluded that cotyledon damage had a negative effect on seedling growth of cotton. Seedlings with one cotyledon removed showed significant reductions in other traits except shoot length and root fresh and dry weight. Root growth was more sensitive to cotyledon damage than shoot growth. When both cotyledons were removed, all the seedling growth parameters were lower than the control. As a result, there would be a significant delay in seedling development and that the plants could tolerate 50% cotyledon damage if all cotyledons were damaged.

REFERENCES

Anonymus, 2024. Ulusal Pamuk Konseyi Pamuk Sektör Raporu, 2023, https://shorturl.at/FyoPq Erişim tarihi: 31.08.2024.

Bisognin, D.A., Velasquez, L., Widders, I., 2005. Cucumber seedling dependence on cotyledonary leaves for early growth. Pesquisa Agropecuária Brasileira, 40: 531-539.

Chen, H., Zhao, M., Qi, L., Sun, X., Li, Q., Liu, X., Wang, N., Zwiazek, J.J., Zhang, W., Guo, W., Wang, R., Zhang, F., Du, N., 2023. Shading reduced the compensation and enhancement effects of soil nutrition on the growth of cotyledon-damaged *Quercus acutissima* seedlings. Plant and Soil, 482(1): 665-678.

Green, J.A., Minton, E.B., 1980. Influence of mechanical damage and fungicide seed treatments on germination and stand with cottonseed. Crop Science, 20(2): 235-239.

Hanley, M.E., Fegan, E.L., 2007. Timing of cotyledon damage affects growth and flowering in mature plants. Plant, Cell & Environment, 30(7): 812-819.

Hanley, M.E., May, O.C., 2006. Cotyledon damage at the seedling stage affects growth and flowering potential in mature plants. New Phytologist, 169(2): 243-250.

Hu, X.W., Zhang, R., Wu, Y.P., Baskin, C.C., 2017. Seedling tolerance to cotyledon removal varies with seed size: A case of five legume species. Ecology and Evolution, 7(15): 5948-5955.

Iortsuun, D.N., Chia, A.M., Adeola, A.F., 2008. The effect of seed mass and cotyledon removal on the germination and growth of fluted pumpkin (*Telfaria occidentalis* Hook. F). Science World Journal, 3(1): 25-31.

Kaya, M.D, Bayramin, S., 2013, Effect of cotyledon leaf damage on plant development in sunflower (*Helianthus annuus* L.), 10th Field Crops Congress, 10-13 September, Konya, Türkiye.

Kennedy, P.G., Hausmann, N.J., Wenk, E.H., Dawson, T.E., 2004. The importance of seed reserves for seedling performance: An integrated approach using morphological, physiological, and stable isotope techniques. Oecologia, 141: 547-554.

Kitajima K., 2003. Impact of cotyledon and leaf removal on seedling survival in three tree species with contrasting cotyledon functions. Biotropica, 35: 429-434.

Moscardi, F., de Freitas Bueno, A., de Freitas Bueno, R.C.O., Garcia, A., 2012. Soybean response to different injury levels at early developmental stages. Ciência Rural, 42(3): 389-394.

Ödemiş, B., Candemir, D.K., Delice, H., Karazincir, K., 2018. Determination of the effects of different water stress levels on yield and vegetative properties of cotton (*Gossypium hirsutum* L.) in Hatay. Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi, 23(1): 58-75.

TUİK, 2024. Turkish Statistical Institute, Plant Production Data, https://biruni.tuik.gov.tr/medas/?locale=tr Accessed date: 31.08.2024.

Wallace, S.K., Eigenbrode, S.D., 2002. Changes in the glucosinolate-myrosinase defence system in *Brassica juncea* cotyledons during seedling development. Journal of Chemical Ecology, 28: 243-256.

Xiao, Z., Zhang, Z., Krebs, C.J., 2013. Long-term seed survival and dispersal dynamics in a rodent-dispersed tree: testing the predator satiation hypothesis and the predator dispersal hypothesis. Journal of Ecology, 101(5): 1256-1264.

DIGITALIZATION OF LOCAL PLANT GENETIC RESOURCES IN THE FRAMEWORK OF MAINTAINING INTEROPERABILITY IN THE EUROPEAN AREA

Nikolaya Velcheva

Agricultural Academy, Institute of Plant Genetic Resources "K. Malkov", Department of Plant Genetic Resources, 2 Druzhba Str., 4122 Sadovo, Plovdiv district, Bulgaria

Corresponding author e-mail: nikolaya_velcheva@abv.bg

ABSTRACT

Plant genetic resources (PGR) play an important role in maintaining global food security, conserving biodiversity and promoting sustainable agriculture. International cooperation between genebanks has become crucial in ensuring the conservation and sustainable use of PGR. The paper aims to evaluate the role of the European collaboration for development of local PGR collection of Bulgaria, based on the long-standing mission of conserving, evaluating and using plant diversity. By sharing experiences, best practices and standardization of the genebank activities the collaboration in ECPGR aims to increase the use of national collections in line with the EU Biodiversity Strategy for 2030. In the area of digitalization, the partnership aims to improve the quality of data management in accordance with the FAO/Bioversity descriptors and to make it free accessible according to the Nagoya Protocol. The results include development of optimized databases supporting equitable sharing of benefits from the use of genetic resources under the International Treaty on PGR for Food and Agriculture. European collaboration contributes to environmental and traditional food protection through valorization of indigenous PGR and their reintroduction to farms in line with the national conservation strategies.

Keywords: Plant diversity, Landrace, Collection, Data base, European collaboration.

INTRODUCTION

Plant genetic resources (PGR) play an important role in maintaining global food security, conserving biodiversity and promoting sustainable agriculture (Halewood et al., 2018). International cooperation between genebanks has become crucial in ensuring the PGR documentation and sustainable use of their biological potential (Tienhaara et al., 2019; Kotni et al., 2023; Shaw et al., 2023). By sharing experiences, best practices and standardization of the genebank activities the EU collaboration aims to increase the use of national collections in connection with the PGR Strategy for Europe (ECPGR, 2021). The program for digitalization of plant biodiversity in the European partnership is focused on improvement the quality of data management system following the FAO standards and to make it free accessible according to the Nagoya Protocol (CDB, 2011) and International Treaty on PGR (ITPGRFA, 2009).

Plant biodiversity is preserved through a global system of national and international genebanks. *Ex situ* storage and collection management requires systematic documentation of passport data, characterization and evaluation information for PGR accessions (Mendel et al., 2019). Maintaining interoperability in the European area is an important prerequisite for the implementation of cooperation between genebanks. Artificial Intelligence have great potential to support the improvement of the processes for access, exchange and use of stored PGR (Doukovska, 2021). In this context, 21st century genebanks are required to provide complete information about germplasm collections to their users – researchers, crop breeders, other stakeholders (Treuren and Hintum, 2014; Weise, 2021).

Open access platforms such as EURISCO (Weise et al., 2017), AEGIS (2022), GENESYS (2015), WIEWS (FAO, 2020) provide centralized international databases with the possibility of searching accessions conserved in genebank collections by standard criteria based on taxonomy, botanical and agronomic characteristics of plant species. The portals have electronic references to the collections in the national genebanks, but still this system has a high level of complexity and requires specific knowledge from the PGR users. In accordance with the principle of "Open Science", it is necessary to take more and more effective actions to make the genebanks information accessible for society, that will increase the valorization of research work, as well as the opportunities to use the potential of the conserved plant gene pool.

The research work aims to evaluate the role of European collaboration for digitalization of Bulgarian genebank collection, based on the long-term mission of conservation, evaluation and use of local PGR in connection with EU Biodiversity Strategy for 2030.

MATERIAL AND METHOD

The Information Center for plant genetic resources at IPGR-Sadovo has been established in 1982 and completely renovated in the period 2021-2023. It works according to the international standard of FAO/Bioversity (2017).

The National Genebank of Bulgaria, situated at IPGR-Sadovo, was built with the financial support from FAO in 1984 and carries out a scientific program for the long-term preservation of germplasm through seeds under controlled conditions.

Annually, expeditions in different geographical regions of the country are conducted by different national and international projects and valuable local varieties and crop wild relatives are collected (Velcheva and Uzundzhalieva, 2022).

The National Genebank is nominated as a focal point in the European Search Catalogue for Plant Genetic Resources – EURISCO (*http://eurisco.ecpgr.org*). EURISCO is an open access network, providing information about *ex situ* plant collections in Europe. Central Crop Databases are *on line* available in the ECPGR cooperation system. Through EURISCO the information about the Bulgarian National Inventory is transferred and accessible in other international databases, such as A European Genebank Integrated System – AEGIS, FAO WIEWS and Electronic Platform for Plant Genetic Resources for Food and Agriculture, Conserved in Genebanks Worldwide – GENESYS (Velcheva et al., 2017).

RESULTS AND DISCUSSION

The program of Department of Plant Genetic Resources in IPGR-Sadovo is responsible for conservation of PGR and representing Bulgaria at international cooperation in ECPGR. The research activity includes:

(1) enrichment of the *ex situ* collections with new germplasm;
(2) documentation by FAO/Bioversity standard;
(3) study, evaluation and characterization by ECPGR descriptors by crop groups;
(4) storage under long-term conditions;
(5) maintenance, propagation and free exchange of PGR;

(6) conservation of crop wild relatives in lively *in vivo* collections;
(7) *in situ/on farm* conservation of wild, semi-wild, local varieties and populations;
(8) sustainable use of PGR.

Information activities are essential part from genebank management system and they are historically presented in the Table 1.

Table 1. Information and documentation activities in Bulgarian Genebank

Before 1982	• field books • no computerization – data management on paper
1982-2000	• Info & Doc center – data management on paper and database • Accession data managed in isolated files • Passport data; C&E data under UPOV descriptor lists
Since 2000	• Phyto 2000 DB in MS Access file format – passport data • C&E data in MS Excel file format under UPOV, IBPGR, ECPGR descriptor lists
2021-2023	• Bulgarian Genebank Documentation System – collaboration between IPGR-Sadovo with Plovdiv University, Department of Computer Science and Institute of Information and Communication Technologies of Bulgarian Academy of Science
2024-2026	• DIGIVALPGR Project – collaboration between Bulgarian and Slovakian genebanks

Data management system Phyto 2000

IPGR-Sadovo has an electronic register PHYTO 2000 in Microsoft access 2003 format (Figure 1). It contains all the information about the seed accessions kept in the genebank. Each accession has a unique number and passport characterization to be clear enough where the accession is – in base collection (long-term) in exchange collection (medium term), work collection, *in vitro*, *in vivo* collections. The data management system is outdated, unsecure and difficult to maintain so it had needed a full reengineering. In the context of the conservation of plant genetic resources, secure and well-structured documentation of the material is essential (Weise et al., 2020).

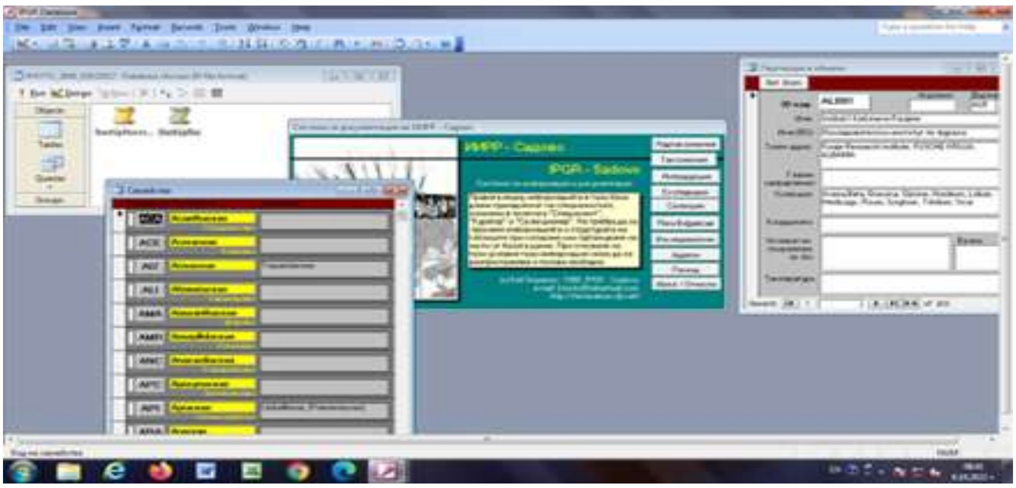


Figure 1. Electronic register PHYTO 2000 of Bulgarian genebank

Bulgarian Genebank Documentation System

In 2023 intelligent documentation system with specialized software, functional ontologies for free access to PGR for all stakeholders and assured security of records through blockchain technologies is established (*https://genbank.uni-plovdiv.net/login*). Starting with the use of field books, a gradual development of electronic data base as national register, nowadays the intelligent data management system aims to improve the availability of conserved seed accessions in genebank to users. The information system was created using Java program in Vaadin Platform. Database is MySQL. The structure of the ontology and metadata are exhibited along with each of the concepts and properties in order to present knowledge about plant genetic resources using the capabilities and benefits of ontologies. The ontology was developed on the basis of the taxonomy for plant genetic resources and the European standard EURISCO. The use of blockchain technologies provides the necessary level of security, completeness and reliability in the input and use of information related to the Bulgarian Genebank. The main tasks in the environment development are the digitalization of the Bulgarian Genebank and the creation of a web portal providing information and services to different groups of users.

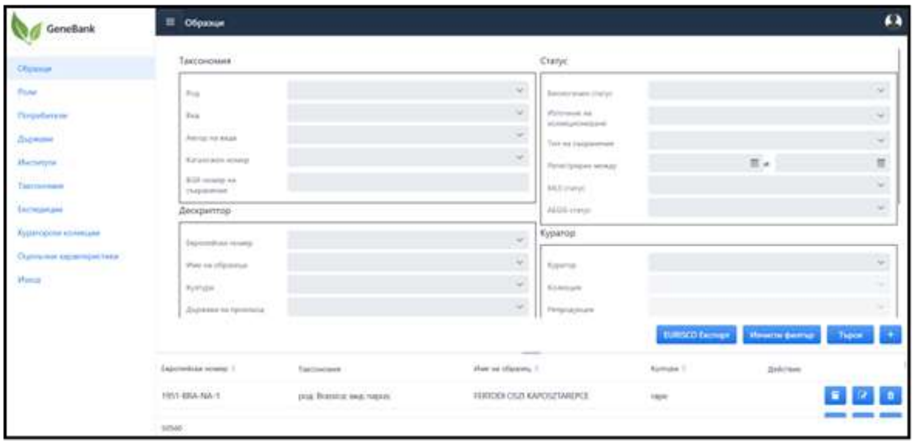


Figure 2. Genebank Bulgaria Documentation System

The Genebank Documentation System present basic information on PGR – accession number, genus, species, name of accession, as well as information about holding institute code, country of origin, status of accessions, source of the material, date of collection, type of storage, whether the genotype has safety duplication in another genebank, etc. Passport descriptors also contain information about wild material received from collection missions such as the collector‘s institute name, collection number, collection name, geographic data and description of the collection site.

Open access of local PGR diversity

As well an electronic platform with free access for local plant gene pool has been created, which includes accessions from traditional crops, used by local communities collected through expeditions (*https://plantsdigcatalog.agriacad.bg/*).

European collaboration on PGR digitalization

The passport data of the Bulgarian genebank is transferred to the global system for plant genetic resources in Europe – EURISCO.

According to EURISCO, data check September 2024, there are 66,399 preserved seed accessions in IPGR-Sadovo from 70,834 totally registered plant germplasm in Bulgaria (*http://eurisco.ecpgr.org*), where 18,846 accessions are with local origin (Table 2).

Table 2. Bulgarian National Inventory in EURISCO

INSTCODE	Holding institute name	Number of accessions	Accessions of Bulgarian origin
BGR001	Institute for Plant Genetic Resources, Sadovo	66,399	16,998
BGR005	Institute of Rose and Essential-oil Plants, Kazanlak	563	4
BGR007	Institute of Maize, Knega	13	13
BGR015	Institute of Forage Crops, Pleven	2	2
BGR029	Dobrudja Agricultural Institute, General Toshevo	3,857	1,829
	Total	70,834	18,846

By signing in 2009 the Memorandum of understanding for the establishment of a European Genebank Integrated System - AEGIS, IPGR-Sadovo participates in the establishment of "virtual" EU genebank with free access collection of unique local germplasm accessions (*http://aegis.cgiar.org*). The status of the Bulgarian AEGIS collection amounts to 391 landraces, including 326 accessions *Triticum sp.*, 15 acc. *Secale sp.*, 9 acc. *Lathyrus sp.* and 41 acc. medicinal plants.

During the last 20 years, there have been significant efforts to intensify cooperation between genebanks in order to sustainable conservation of plant biodiversity. Information networks have been established and they have been an important basis for the development of international systems such as GENESYS and FAO WIEWS, which serve as central entry points for the search for plant genetic resources.

In order to meet the requirements of the innovative research landscape, which is characterized by constant digitalization and big data, the information systems of the genebanks have to be able to make their data free available. The free access to plant biodiversity leads to Open science to new knowledge about plant genetic resources, which ultimately improves their usability and the reputation of the genebanks.

The free access of Bulgarian National Inventory data is following the program for digitalization and smart agriculture in Bulgaria. Nevertheless, Bulgaria is quite behind from other EU members in regards to introduction of digital technologies in the economy and society taking one of the last places in EU in terms of Integral Index for Introduction of Digital Technologies in the Economy and Society (Bachev, 2020).

CONCLUSIONS

In this research, an overview of the used documentation systems and EU cooperation of Bulgarian Genebank was given.

Databases supporting public access and equitable sharing of benefits from the conservation of plant gene pool under the International Treaty on PGR for Food and Agriculture and Nagoya Protocol were established.

At international level, EU plays an active role, contributing to ensure the achievement of the global appointments for biodiversity and PGR conservation.

The main goal of the EU programs is to improve the coordination in conservation activities in Europe and to facilitate the access to PGR and the information about them.

Digitalization of plant genetic resources assist the transfer, use, and dissemination of research results for sustainable agriculture.

ACKNOWLEDGMENTS

This research work was carried out with the support of Bulgarian National Science Fund under the project DIGIVALPGR “Digitalization and valorization of local plant genetic resources in Bulgaria and Slovakia in the framework of maintaining compatibility and interoperability in the European area”, Contract number KP-06-Slovakia/9/13.08.2024.

REFERENCES

AEGIS (2022) Genebank Review. IPGR-Sadovo, Bulgaria, October 27th, 2022.https://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/AEGIS/PEER_REVIEWS/GBank_Review_IPGR-Sadovo.pdf

Bachev H. (2020) Digitalisation of Bulgarian agriculture and rural areas. Ikonomika i upravlenie na selskoto stopanstvo. Bulgarian Journal of Agricultural Economics and Management, 65(2), 3-24.

CDB (2011) Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity. https://www.cbd.int/abs/doc/protocol/nagoya-protocol-en.pdf.

Doukovska L. (2021). Artificial Intelligence to Support Bulgarian Crop Production. Engineering Sciences, Vol. LVIII, No 4, 30-48, doi: 10.7546/EngSci.LVIII.21.04.03.

ECPGR (2021) Plant Genetic Resources Strategy for Europe. Rome, Italy. https://www.ecpgr.cgiar.org/fileadmin/bioversity/publications/pdfs/PGR_STRATEGY_LP_22_Nov_revised.pdf.

FAO (2020). World Information and Early Warning System on Plant Genetic Resources for Food and Agriculture (WIEWS). http://www.fao.org/wiews/.

FAO/Bioversity (2017) Multi-Crop Passport Descriptors, Rome, Italy. https://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/EURISCO/EURISCO_MCPD2_descriptors_updated_November_2017.pdf.

GENESYS (2015) The Global Gateway to Genetic Resources. https://www.genesys-pgr.org/.

Halewood M., Chiurugwi T., Sackville Hamilton R., Kurtz B., Marden E., Welch E., Powell W. (2018) Plant genetic resources for food and agriculture: opportunities and challenges emerging from the science and information technology revolution. New Phytologist, 217(4), 1407-1419.

ITPGRFA (2009) International Treaty on Plant Genetic Resources for Food and Agriculture. Rome, Italy. https://www.fao.org/plant-treaty/en/.

Kotni P., Hintum T. van, Maggioni L., Oppermann M., Weise S. (2023) EURISCO update 2023: the European Search Catalogue for Plant Genetic Resources, a pillar for documentation of genebank material, Nucleic Acids Research, Volume 51, Issue D1, D1465–D1469.

Mendel, Ľ., Hauptvogel, P., Čičová, I. (2019). Plant genetic resources information system of Slovakia as the primary source of the information. Agrobiodiversity for improving of nutrition, health and quality of life, No. 3 Nitra; Kyiv: SPU; pp. 178-185. https://doi.org/10.15414/agrobiodiversity.2019.2585-8246.178-185.

Shaw P. D., Weise S., Obreza M., Raubach S., McCouch S., Kilian B., Werner P. (2023) Database solutions for genebanks and germplasm collections. In Plant genetic resources for the 21st century pp. 285-309. Apple Academic Press.

Tienhaara A., Ahtiainen H., Pouta E., Czajkowski M. (2019). Role of information in the valuation of unfamiliar goods - the case of genetic resources in agriculture. Working Paper-Faculty of Economic Sciences, University of Warsaw, WP7/292.

Treuren van R., Hintum van T. (2014) Next-generation genebanking: Plant genetic resources management and utilization in the sequencing era. Plant Genetic Resources, 12(3), 298-307.

Velcheva N., H. Knüpffer, S. Weise (2017) Bulgarian National Inventory in International Plant Genetic Resources Databases. Proceeding of International conference „135 years Agricultural Science in Sadovo and 40 years Institute of Plant Genetic Resources". 137-144.

Velcheva N., Uzundzhalieva K. (2022) Status of plant genetic resources for food and agriculture in Bulgaria: from genotype collecting to information access. In Proceedings of IV. International agricultural, biological & life science conference AGBIOL 2022, p. 177-186.

Weise S. (2021). Data management for preserving genetic diversity: Experiences and challenges. 34th Meeting EUCARPIA Fodder Crops, 12-16, DOI: 10.5507/vup.21.24459677.

Weise S., M. Oppermann, L. Maggioni, T. van Hintum, H. Knüpffer (2017) EURISCO: The European search catalogue for plant genetic resources. Nucleic Acids Research. 45 (Database issue). D1003-D1008.

Weise, S., Lohwasser, U., Oppermann, M. (2020) Document or lose it—on the importance of information management for genetic resources conservation in genebanks. Plants, 9(8), 1050.

DIVERSE PLANT GENETIC RESOURCES FOR SUSTAINABLE FOOD CHAINS IN BULGARIA

Nikolaya Velcheva, Tsvetelina Stoilova, Valko Neshev

Agricultural Academy, Institute of Plant Genetic Resources "K. Malkov", Department of Plant Genetic Resources, 2 Druzhba Str., 4122 Sadovo, Plovdiv district, Bulgaria

Corresponding author e-mail: nikolaya_velcheva@abv.bg

ABSTRACT

The value of biodiversity for food security and dietary balance has been underlined as priority by FAO and Bioversity International. The study aims to evaluate the underutilized local Plant Genetic Resources (PGR) in Bulgaria and to extensively document the traditional food and seed systems in which PGR are used in order to promote their use for the benefit of farmers and consumers. The two basic concepts related to this general objective are: (1) to retrieve the embedded knowledge of local communities, in order to enhance awareness on PGR values; and (2) to support a chain-perspective approach in PGR study and management, aimed at development of *on farm* conservation. The results include harmonizing concepts of PGR international treaties; enrichment of current PGR collections; enhancing the documentation of the databases; prompting the urban consumers' awareness on the value of PGR for nutrition, biodiversity and sustainable agriculture. Main outcome is the creation of thematic networks and knowledge base aimed at promoting the durable use of local PGR and *on farm* conservation.

Keywords: Genebank, Accession, Expedition, *On farm* conservation, Climate change.

INTRODUCTION

The modernization of agriculture and the spread of intensive cultivars led to processes that replaced the rich diversity of traditional varieties. Farmers are motivated to grow high-yielding cultivars to maximize their income. At the same time, in a competitive market environment, breeding companies produce seeds only of those varieties and crops that they trust will generate profits for them and promote their new cultivars with a rich arsenal of marketing techniques. However, the fact is that climate change is changing the environmental conditions and more and more farmers are looking for sustainable yields and a bigger assortment of varieties and crops to guarantee income even in less favorable conditions (Reidsma et al., 2010; Villanueva et al., 2017; Borrell et al., 2019). Therefore, it is very necessary to protect biodiversity – landraces that has evolved in local agricultural systems (Shelef et al., 2017).

The value of biodiversity for sustainable agriculture, food security and dietary balance has been underlined as priority by EU Biodiversity Strategy for 2030 (EU, 2020a). The efforts to conserve PGR *ex situ* in the genebank are prevalent to guarantee plant diversity preservation (Scarascia-Mugnozza and Perrino, 2002). Integrating *ex situ* and *in situ/on farm* conservation is the best approaches in the management of local diversity (Engels and Ebert, 2021). The *on farm* conservation of local varieties is the most effective activity to preserve their specific characteristics in the specific agro-climatic conditions that formed this diversity. It is imperative to establish close links between local farmers and researchers to achieve an integrated model for PGR conservation (Knüpffer, 2002; Arndorfer et al., 2009; Galluzzi et al., 2010).

According to Knüpffer (2016), the Balkan Peninsula is characterized by a very high level of plant biodiversity. The geographical position, climatic conditions and relief determines a rich species diversity, well adapted to the agro-ecological local regions. Preliminary survey for mapping the distribution of local varieties in Bulgaria by using the national register of plant genetic resources was done from Velcheva (2022).

Valorization encompasses the process by which plant landraces become a symbol, playing an important role in the construction of local identity and the specific flavor of a certain area (Ganeva-Raycheva et al., 2018; Britwum and Demont, 2022). The European Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system support this goal as a priority policy of EC (2020b).

The study aims to evaluate the status of local plant genetic resources (PGR) in Bulgaria and to extensively document the traditional food and seed systems in which PGR are used in order to promote their use for the benefit of farmers and consumers.

MATERIAL AND METHOD

The research activities of Department of Plant Genetic Resources in IPGR-Sadovo part from Agricultural Academy are mainly aimed at collection, documentation, study, conservation and sustainable use of plant diversity of Bulgaria. In the Institute is situated the National Seed Genebank, which stores long-term local and introduced accessions according to the Standards of FAO (2014). Enrichment of *ex situ* collections with new PGR is carried out through expeditions, international free germplasm exchange and storage of breeding materials, deposited from Bulgarian Institutes of the Agricultural Academy and similar scientific organizations of the country. The collecting missions for enrichment the genebank with local varieties and populations is according to the Guidelines of FAO (2019). The inventory plan includes the following information: farmer's variety/landrace names, farmer information, site data, crop data, socio-economic data. The survey includes characterization and evaluation data. *On farm* conservation activities are according to Veteläinen et al., (2009) and the Concept of ECPGR (2017).

RESULTS AND DISCUSSION

Local germplasm collecting activities were first initiated from N.I. Vavilov and his colleagues at the Russian Institute of Plant Genetic Resources (VIR) during the 1920s and 1930s. It was also based on the concerning evidence emerging in the mid-1930s that traditional crop varieties and adapted landraces were being replaced by new improved varieties. In the 1970s *ex situ* collections were promoted by international institutions, in recognition of the importance of plant genetic resources for global food security. FAO and Bioversity International play key roles in this regard.

Two basic hypotheses are related to the general objective of the local diversity sustainable conservation and use: (1) to retrieve the embedded knowledge of local communities, in order to enhance awareness on PGR values; and (2) to support a chain-perspective approach in PGR study and management, aimed at development of *on farm* conservation.

Inventory of local PGR collection, stored in the National Genebank

During the period from 1982 to August, 2024, 11,083 local accessions have been collected from home gardens and small farms, as well as crop wild relatives from their natural habitats, and conserved at the genebank of IPGR-Sadovo. The status of collection and the genera with the biggest number of local accessions in Bulgarian genebank is presented on the Figure 1. As a result of enrichment, collections were enriched with high number of farmer's variety/landrace from the genus *Phaseolus*, *Capsicum*, *Allium*, *Cucurbita*, *Lycopersicon*, *Vicia*, etc. Local varieties originating from Bulgaria compares 28% of the fund of National Genebank.

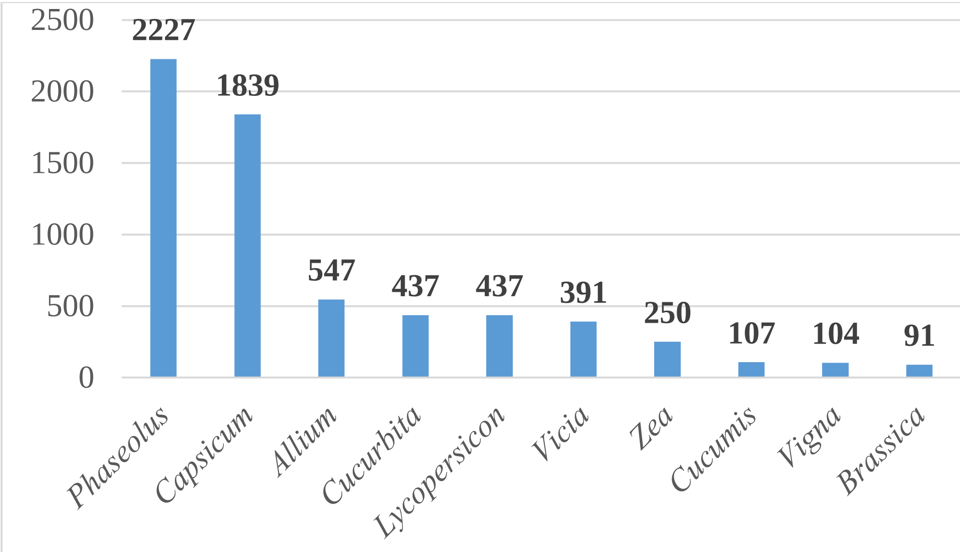


Figure 1. Crops with the biggest number of local accessions in Bulgarian genebank

Socio economic analysis of PGR-based local food chain systems and traditional products

As a result of the explored datasets of the National register of PGR it was found that local diversity grown in rural areas of Bulgaria is still characterized by a large number of crops: grain legumes, vegetables, medicinal species. The role of access to information on conserved plant biodiversity for achieving sustainable development of the regions can be considered in several directions – their participation in the agro-food chain, for production of traditional products, provide varietal diversity in local markets, as well as in rural tourism. Traditional varieties have potential in relation to return the interest of people to rural areas, to increase hobby gardening and organic production. The role of documentation and access to local PGR comes into focus on the biological, agricultural, social and economic prospects for use of these resources and traditional knowledge related to their cultivation, a time when society is sensitive to ecology and challenges, caused by climate change, with a growing need for higher yields and more well adapted crops.

Such is the example of traditional large beans, sweet diverse forms peppers and tomato “heart” type, which were not subject to intensive cultivation and wide commercialization, now they become a value, a "trademark" and are associated with the name of a settlement. This is the result of complex factors, economic and socio-cultural prerequisites, and is an example of how valorization can be a stimulus for the activation of local communities, the revival of traditions in the region of origin and the development of agritourism in localities. It is the sustainable storage of these valuable resources and access to passport, characterization and evaluation information that is at the heart of preventing their loss and preserves the possibility of their return to the agro-food chain in the region of origin.

From August, 2024 IPGR-Sadovo implements 24 months DIGIVALPGR project. The specific challenge is to promote the use of farmer's variety/landrace as traditional resources for agricultural diversity and the agro-food chain through research and innovative technology following different tasks and activities (Figure 2).

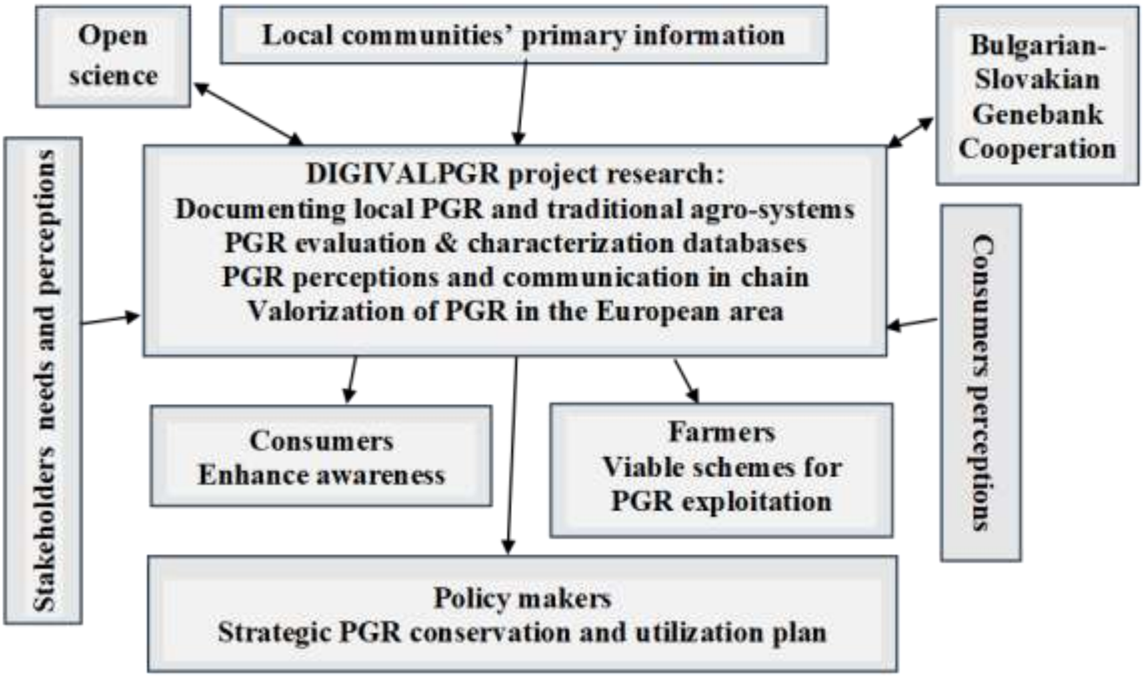


Figure 2. DIGIVALPGR project research tasks and activities

The regional food chains will be analyzed considering: 1) the role of local systems in preserving food security; 2) the role of economic valorization of local systems in preserving local PGR; 3) the role of economic valorization of local systems in improving capacities and infrastructures at local level; 4) social effects, deriving from the economic valorization of local food systems.

Documentation and conservation approaches of DIGIVALPGR project

- **Development of a farmer's variety/landrace database** in partnership with Slovakian genebank, based on the existing general European plant germplasm catalogue (EURISCO). The farmer's variety/landrace register is envisaged to function as a bio-cultural heritage information resource.
- **Collecting of landraces and crop wild relatives**. Collecting missions in Bulgaria and Slovakia will be set up for the selected traditional crops. The inventarization data will be stored in the national information systems, that will be uploaded in European catalogue EURISCO and global plant genetic resources system GENESYS.
- **Description and survey of the farmer's variety/landrace**. This activity consists of the characterization and evaluation of the varieties, collected by ECPGR descriptor lists.
- **Conservation of seed and free access**. The regenerated seeds from collected accessions during the expeditions will be stored in the genebank, according to best practices for *ex situ* management of collections and included in the exchange collection by SMTA (Standard Material Transfer Agreement).
- **Dissemination of results according to the Open science principals**.

A questionnaire for collecting data during the expeditions was created, according to ECPGR (2017) and Guidelines of FAO (2019):

QUESTIONNAIRE for collecting data during the collecting missions for farmer's variety/landrace by DIGIVALPGR project	
1	Accession number
2	Collection date
3	Researcher who collected the accessions
4	Collection site/place 1) Country/city/village/locality 2) Latitude 3) Longitude 4) Altitude 5) Characteristics of the area - flat, mountainous, semi-mountainous, etc. 6) Climatic features - temperatures, precipitation during the growing season
5	Farmer information 1) Name and surname 2) Age 3) Main activity
6	Crop/taxonomic description
7	Local accession name
8	Biological status 1) Wild / Crop wild relatives 2) Local / traditional variety, population 3) Old variety (with reduced use)
9	Characteristics of the accession 1) Agronomic - cultivation technology, early maturity, yield, etc. 2) Product quality – shape, color, taste, etc. 3) Resistance to diseases, enemies, drought, etc. 4) Irrigated / non-irrigated conditions 5) Specific features – distinguishing qualities and signs 6) Where the accession was obtained from and how long it has been grown on the farm
10	Farm information 1) Total area of used agricultural land in the farm 2) Area on the farm occupied by the accession 3) Reasons for growing the accession – traditional, cultural/religious, market, palatability, early maturity, disease resistance, etc. 4) Direction of production – for domestic consumption, local market, ecotourism, for the country/abroad, direct market/through an intermediary, etc. 5) How do they get seeds - do they collect themselves, from neighbors or from the local market
11	Traditional recipes for using the accession and other additional information
12	Photo of accession

CONCLUSIONS

European collaboration through different initiatives contributes to traditional food protection through valorization of landraces and their reintroduction to agricultural practice in correspond with the international conservation strategies.

ECPGR has brought genebanks together to exchange experiences, draft common descriptor lists, set up various joint projects and improve their documentation developing strong working relationships and initiatives supporting *ex situ* and in situ/on farm PGR community.

Local varieties originating from Bulgaria consist 28% of the fund of National Genebank, but there is opportunity to increase their part through collecting missions in rural areas by DIGIVALPGR project.

The socio-economic analysis identifies the strengths and critical issues related to the exploitation of genetic resources in local/regional agri-food systems. The analysis informs policy-makers on the activity options that can support agro-biodiversity conservation and foster economic development in current climate change conditions.

ACKNOWLEDGMENTS

This research work was carried out with the support of Bulgarian National Science Fund under the project DIGIVALPGR "Digitalization and valorization of local plant genetic resources in Bulgaria and Slovakia in the framework of maintaining compatibility and interoperability in the European area", Contract number KP-06-Slovakia/9/13.08.2024.

REFERENCES

Arndorfer M., B. Kajtna, B. Vorderwülbecke (2009) Integrating *ex situ* and *on farm* conservation approaches in the management of local vegetable diversity in Austria. Acta Hortic. 817, 333-340.

Borrell, J. S., S. Dodsworth, F. Forest, O. A. Pérez-Escobar, M. A. Lee, E. Mattana, P. C. Stevenson, M.-J. R. Howes, H. W. Pritchard, D. Ballesteros, B. Kusumoto, I. Ondo, J. Moat, W. Milliken, P. Ryan, T. Ulian, S. Pironon (2019) The climatic challenge: Which plants will people use in the next century? Environmental and Experimental Botany. ISSN: 0098-8472. Page: 103872.

Britwum K., Demont M. (2022) Food security and the cultural heritage missing link. Global Food Security, 35, 100660.

EC (2020a) EU Biodiversity Strategy for 2030. Bringing nature back into our lives. Communication for the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the regions, p-25.

EC (2020b) A Farm to Fork Strategy for a fair, healthy and environmentally-friendly food system: https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1590574123338&uri=CELEX:52020DC0380

ECPGR. (2017) ECPGR Concept for *on-farm* conservation and management of plant genetic resources for food and agriculture. Rome, Italy. https://www.ecpgr.cgiar.org/fileadmin/bioversity/publications/pdfs/ECPGR_Concept_for_on_farm_final__05_05_2017_bis.pdf

Engels J., Ebert A. (2021) A critical review of the current global *ex situ* conservation system for plant agrobiodiversity. I. History of the Development of the Global System in the Context of the Political/Legal Framework and Its Major Conservation Components. Plants, 10, No. 8: 1557. doi: 10.3390/plants10081557.

FAO (2014) Genebank standards for plant genetic resources for food and agriculture. Rome. Italy.

FAO. (2019) Voluntary Guidelines for the Conservation and Sustainable Use of Farmers' Varieties/Landraces. Rome, Italy. http://www.fao.org/3/ca5601en/ca5601en.pdf

Galluzzi G., P. Eyzaguirre, V. Negri (2010) Home gardens: Neglected hotspots of agro-biodiversity and cultural diversity. Biodiversity and Conservation. 19(13). 3635-3654.

Ganeva-Raycheva V., Dimitrova D., Ivanova T. (2018) Plants and Identity: from Biocultural Characteristics to Valorization of Local Plant Resources. Bulgarian Folklore. Vol. XLIV, Issue 3, 361-389.

Knüpffer H. (2016) Plant genetic resources from the Balkan Peninsula in the world,s genebanks. Journal of Agriculture, Food and Enveronme.Sciense. Vol. 69. 53-68.

Knüpffer H. (2002) Documentation of plant genetic resources in home gardens. Home gardens and in situ conservation of plant genetic resources in farming systems. 19.

Reidsma P., F. Ewert, A. Oude Lansink, R. Leemans (2010) Adaptation to climate change and climate variability in European agriculture: The importance of farm level responses. European Journal of Agronomy. Vol. 32, Issue 1. 91-102.

Scarascia-Mugnozza G. T., Perrino P. (2002) The history of *ex situ* conservation and use of plant genetic resources. In Managing plant genetic diversity. Proceedings of an international conference, Kuala Lumpur, Malaysia, 12-16 June 2000, pp. 1-22. Wallingford UK: Cabi Publishing.

Shelef O., Weisberg P. J., Provenza, F. D. (2017) The value of native plants and local production in an era of global agriculture. Frontiers in plant science, 8, 2069.

Velcheva N. (2022) Preliminary survey for mapping the distribution of local varieties by using the national register of plant genetic resources in Bulgaria. Proceedings of International Agricultural, Biological & Life Science Conference AGBIOL 2022. 451-457.

Veteläinen M, Negri V and Maxted N. (2009) European landraces *on-farm* conservation, management and use. Bioversity Technical Bulletin No. 15. Bioversity International, Rome, Italy.

Villanueva A. B., M. Halewood, I. L. Noriega (2017) Agricultural Biodiversity in Climate Change Adaptation Planning. European Journal of Sustainable Development. 6. 2. 1-8.

THE CONTROL POSSIBILITIES OF Fusarium proliferatum, THE AGENT OF ROOT AND CROWN ROT IN PUMPKIN, WITH PROPOLIS EXTRACT (Api10)

Ömer Ümit OKÇU[1], Nuray ÖZER[1], Nagehan Desen KÖYCÜ[1]

[1] Tekirdağ Namık Kemal University, Agricultural Faculty, Plant Protection Department, Tekirdağ, Türkiye

Corresponding author e-mail: ouokcu@nku.edu.tr

ABSTRACT

Propolis produced by honeybees is known to possess antifungal properties against fungal pathogens that cause yield losses in cultivated plants. This has created the opportunity to use propolis as an alternative application to pesticides, which are harmful to the environment and ecological balance. This study investigates the effect of propolis extract (Api10), developed by Tekirdağ Namık Kemal University Technopark (Tekirdağ/Türkiye), on *Fusarium proliferatum*, which causes root and root crown rot in pumpkin (*Cucurbita pepo* L.), a commonly grown plant in Türkiye. Initially, the effects of the extract at different concentrations on the mycelial growth and daily growth rate of the pathogen on Potato Dextrose Agar (PDA) were examined. After seven days of incubation, mycelial growth of the pathogen was inhibited by over 70% at the last three concentrations (0.925, 1.025, 1.125%), with the minimum inhibitory concentration (MIC) being 1.125%. The effective concentration for 50% inhibition of mycelial growth (EC_{50}) of the extract was determined to be 0.335%. While the growth rate in control plates without extract was 1.23 cm/day, no growth was observed at the 1.125% concentration, and the growth rates at 0.925% and 1.025% concentrations were 0.35 and 0.21 cm/day, respectively. Three concentrations found effective against the pathogen's mycelial growth were applied to pumpkin seeds along with the pathogen, and disease severity was measured in seedlings after a one-week incubation period. Disease severity was most effectively inhibited by concentration of 1.125% with a reduction rate of 44.13%. The authors suggest that the propolis extract, whose antifungal effect as seed treatment in pumpkin against *F. proliferatum* is identified for the first time in this study, is promising for integration into pot and field trials.

Keywords: Pumpkin (*Cucurbita pepo*. L.), Propolis extract, *Fusarium proliferatum*, Antifungal effect, Seed treatment

INTRODUCTION

Propolis produced by honey bees has a rich composition that may vary depending on the bee species, collection time, region and plant (Marcucci, 1995; Ghedira et al., 2009; Haile and Dekebo, 2013; Toreti et al., 2013). Propolis is composed of 50-70% balsam and resin, 30% beeswax, 10% essential oils, 5-10% pollen and 5% sugar, as well as vitamins (B, C, E), minerals, phenolic acids and flavonoids. In addition to the aforementioned compounds, it contains a variety of other chemical substances, including aldehydes, ketones, alcohols, aromatic compounds, steroids, inorganic compounds and amino acids (Easton-Calbaria et al., 2019; Bankova et al., 2001; Hunag et al., 2014; Escriche and Juan-Borras, 2018; Bayram et al., 2020; Salatino and Salatino, 2021). The chemical properties of propolis make it an important component of human health (Marcucci, 1995; Tosi et al., 2006; Toreti et al., 2013; Araujo et al., 2016). Furthermore, given the extensive use of fungicides for the prevention of fungal diseases in agricultural crops, propolis is being investigated as a potential alternative for the management of plant diseases, with the aim of protecting the environment and human health. In this context, the majority of propolis-related tests have been conducted *in vitro* conditions, with a focus on the ability of propolis to inhibit conidial germination (Ghaly et al., 1998; Mattiuz et al, 2015) and mycelial development (Quiroga et al., 2006; Meneses et al., 2009; Yang et al., 2011; Haile and Dekebo, 2013; Mattiuz et al., 2015; Özyiğit et al., 2018; Dudoit et al., 2020; Çakar et al., 2022; Ouahab et al. 2023). In some studies, the values for the effective concentration for 50% inhibition of mycelial growth (EC_{50}) or minimum inhibitory concentration (MIC) were determined (Quiroga et al., 2006; Mohammadzadeh et al., 2007; Curifuta et al., 2012; Moreno et al., 2020; Ristivojevic et al., 2020). The pathogenic fungal species used in these tests included; *Alternaria alternata, Aspergillus carbonarius, A. flavus, A. niger, Botryodiplodia theobromae, Botrytis cinerea, Colletotrichum gleosporioides, C. musae, Fusarium equiseti, F. oxysporum, F. proliferatum, F. solani, F. sporotrichiodes, F. subglutinans, F. verticillioides, Monilia fructigena, Penicillum digitatum, P. expansum, P. italicum, P. notatum* and *Trichoderma reesei*. Quantification of the inhibitory effects of propolis on fruit infection by several pathogens has been conducted in limited number of studies. These include studies on quince by *M. fructigena* (Özyiğit et al., 2018), on banana by *C. musae* (Dudoit et al., 2020), and on raspberry by *Penicillum* spp., *A. alternata*, *A. carbonarius* and *B. cinerea* (Moreno et al., 2020).

Fusarium proliferatum, a polyphagous species with a wide host range, is among the pathogens that cause significant yield reduction (Logrieco et al., 1995; Desjardins et al., 1997; Abdalla et al., 2000; Pérez et al., 2011; Yamazaki et al., 2013; Ren et al., 2015; Borrero et al., 2019; Yu et al., 2021; Sevinç and Özer, 2023). A recent study (Demir et al., 2023) found that 51.07% of pumpkin plants exhibited root and crown rot as a result of infection by *F. proliferatum.* No fungicide is currently registered for use in the treatment of the disease. The objective of this study was to investigate the antifungal effect of Api10 propolis extract, developed by Tekirdağ Namık Kemal University Technopark (Tekirdağ/Türkiye), on *F. proliferatum.*

MATERIALS AND METHODS

Materials

The pathogenic fungus used in the study was *Fusarium proliferatum* (FusP9), an isolate obtained by Demir et al. (2023) from naturally infected pumpkin (*Cucurbita pepo* L.) seeds. The TG38 pumpkin cultivar, which has been demonstrated to be susceptible to the pathogen (Sevinç and Özer, 2023), was supplied from AYFA Tarım AŞ. The propolis extract Api10 is a product of Tekirdağ Namik Kemal University Technopark, having a patent (Patent application number 2020/05364; inventor Prof. Dr. İbrahim PALABIYIK, Tekirdağ Namik Kemal University, Faculty of Agriculture, Department of Food Engineering, Tekirdağ/Türkiye)

Methods

In the *in vitro* assays, propolis extract was added to 100 ml Potato Dextrose Agar (PDA) medium at 50^{o}C in an erlenmeyer flask at nine different concentrations (0.325%, 0.425%, 0.525%, 0.625%, 0.625%, 0.725%, 0.825%, 0.925%, 1.025%, 1.125%) and poured into 8.5 cm diameter sterile Petri dishes. Once the medium had solidified, a 7 mm diameter agar disc, from the pathogen culture grown in PDA medium at 25°C in the dark for seven days, was put in the centre of the Petri dish. A control group was constituted by

Petri dishes lacking propolis extract and consisted solely of an agar disc containing the pathogen. The petri dishes incubated at 25ºC in the dark. Following the completion of mycelial colony development in the control, the diameter of the pathogen's mycelial growth was measured in the treated Petri dishes. The precentage of mycelial growth inhibition (MGI%) was calculated as MGI: [(mc-ma)/mc × 100], where mc is the mean of mycelial growth diameter in control and ma is the mycelial growth diameter in treatment (Deans and Svaboda, 1990). Additionally mycelial growth diameter were measured every 48h in petri dishes containing the ranges of concentrations differing from 0.625% to 1.125% (concentrations that inhibit mycelial growth by more than 70) of propolis extract and in control. Mycelial growth rate (cm/day) was calculated. The experiments were desigined in a randomized plot design with five replications (one petri dishes per replicate). The EC_{50} (the effective concentration for 50% inhibition of mycelial growth) value of the propolis extract was determined by applying the percentage growth value calculated according to the control to log-probit paper (Delen et al., 1984). The concentration at which no development of the pathogen was observed yielded the MIC (Minimum Inhibitory Concentration) value.

In order to ascertain the impact of propolis extract on disease severity in seedlings, the concentrations that inhibited mycelial development by over 70% were employed. The seeds were subjected to a seven-minute immersion in a 2% solution of sodium hypochlorite (NaOCl), followed by three rinses with sterile distilled water and a subsequent 30-minute drying period on sterile blotting paper. Seeds that had been subjected to surface disinfection were placed in a mixture of propolis extract and a suspension of *F. proliferatum* conidia (1 x 10^6 conidia/ml) and shaken at 25°C for one hour. A conidia suspension of the pathogen only, in which 0.1% Tween 20 was added, was treated to control seeds. The treated seeds were placed in 8 cm diameter Petri dishes containing PDA, with eight seeds placed in each dish. The experiment was conducted in 13 replicates, with one Petri dish included in each replicate. The seedlings were evaluated using the 0-4 scale (0: Healthy, well-developed seedling, 1: Roots well developed, brown spots on root tip; 2: Browning of root tip, as well as poor root development; 3: Complete browning of roots; 4: No germination and seed completely colonised by fungus) (Figure 1) obtained by modifying the 0-5 scale defined by Demir et al. (2023). Disease severity was calculated using the formula described by Townsend and Heuberger (1943): Disease severity, %: [∑(Disease level x number of seed showing disease at that level)]/[total number of seed × highest severity level × 100]. The efficacy of propolis extract was calculated based on Abbott Formula [% effectiveness C-P)/ C × 100], where in C refers to disease severity in control and P refers to disease severity in the treatment.

Statistical Analysis

The homogeneity of variances and normality of distribution were firsty determined by Levene’s test and Shapiro-Wilk test , respectively, using SPSS, version 23 (IBM SPSS Statistics for Windows, IBM Cora., Armonk, Ny, USA). All in the experiments satisfied the assumptions of ANOVA test. The least significant Difference (LSD) test was used to compare means of treatments at p=0.05 level using Software JMP Version Pro 17 for Windows (Cary, North Carolina, USA).to those of the treatment.

RESULTS AND DISCUSSION

In the study, propolis extract was employed at nine different concentrations, and it was observed that the pathogen did not exhibit growth in the medium containing 1.125% propolis extract, resulting in 100% growth inhibition (Figure 2). This dose was considered to be the MIC value of the extract. The development of the pathogen was observed at concentrations lower than this concentration, but the diameter of the mycelial colony at all doses was significantly lower than in the control (Table 1). Following the concentration of 1.125%, the lowest mycelial growth diameter (1.28 cm) was observed at the 1.025% concentration, which was then succeeded by the 0.925% concentration (2.30 cm). These concentrations inhibited the mycelial growth diameter by 84.94% and 72.94%, respectively. The EC_{50} value of the extract was determined to be 0.335%.

Figure 1. 0-4 scale used in the study. 0: Healthy, well-developed seedling, 1: Roots well developed, brown spots on root tip; 2: Browning of root tip, as well as poor root development; 3: Complete browning of roots; 4: No germination and seed completely colonised by fungus

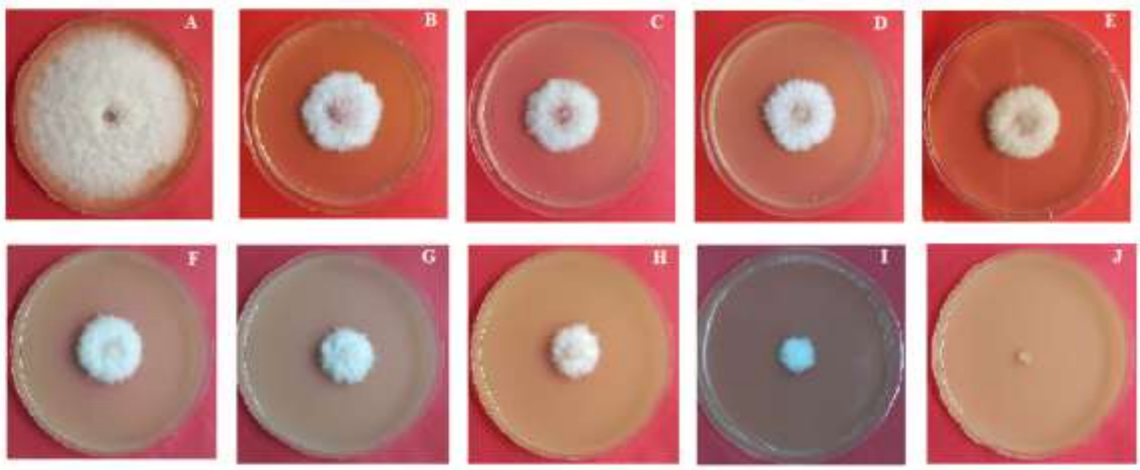


Figure 2. Mycelial growth of *Fusarium proliferatum* in the increasing concentrations of propolis extract (A: Control, B: 0.325%, C: 0.425%, D: 0.525%, E: 0.625%, F: 0.725%, G: 0.825%, H: 0.925%, I: 1.025%, J:1.125%)

Table 1. Effect of different concentrations of propolis extract on mycelial growth of *Fusarium proliferatum*

Concentration (%)	Mycelial Colony Diameter (cm)	Inhibition (%) of mycelial growth
0.325	4.26±0.06b	49.88±0.86
0.425	3.87±0.07c	54.47±0.86
0.525	3.62±0.05d	57.41±0.63
0.625	3.60±0.06d	57.64±0.77
0.725	3.12±0.02e	63.29±0.30
0.825	2.57±0.01f	63.76±0.14
0.925	2.30±0.05g	72.94±0.59
1.025	1.28±0.09h	84.94±1.03
1.125	0.00±0.00i	100.00±0.00
Control	8.50±0.00a	-

*Each value is the mean ± standard error (SE) of five replications. Means in each column followed by different letters differ significantly at P<0.05 according to LSD test.

In the assays where in the growth rate of the pathogen was evaluated, it was observed that the growth rate was 1.23 cm/day in the control Petri dishes (Figure 3). However, a statistically significant reduction in growth rate was observed at concentrations ranging from 0.625% to 1.125%, with no growth occurring at the 1.125% concentration. Furthermore, the growth rates were found to be markedly low at concentrations of 1.025% (0.21 cm/day) and 0.925% (0.35 cm/day).

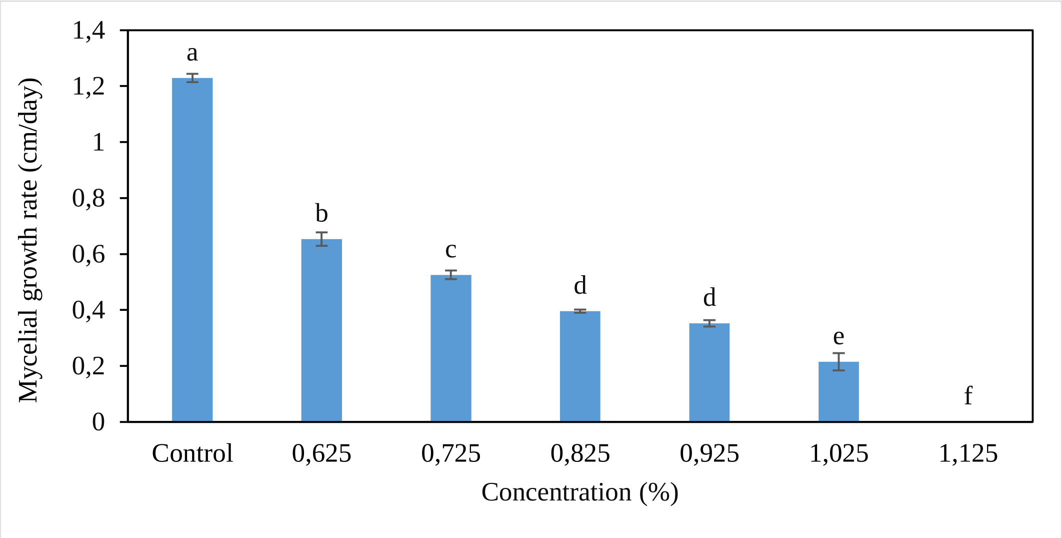


Figure 3. Mycelial growth rate of *Fusarium proliferatum* in different concentrations of propolis extract. Values are means ± standard error (SE) and bars shows SE. Bars topped by a different letter differ significanty based on the LSD test at P<0.005

A review of the scientific literature revealed only one previous study that examined the effect of propolis extract on the mycelial development of *F. proliferatum*. The related study examined the effect of an ethanol extract of propolis on *F. proliferatum* isolated from anise seed, determining the MIC value to be 0.004 mg/ml (Rostivotevic et al., 2020). The isolate *F. proliferatum* was obtained from pumpkin seeds in the current study, and the minimum inhibitory concentration (MIC) of the non-alcoholic propolis extract was found to be 1.125% for this isolate. In a study conducted by Quiroga et al. (2006), it was reported that propolis extracted with ethanol at a concentration of 1.16 mg/ml demonstrated a 55.6% inhibition of the colony diameter of *F. oxysporum*. In a separate study (Ouahab et al., 2023), it was proposed that acetone-extracted propolis (APE2) exhibited inhibitory effects on the colony diameter of the same species, with an inhibition rate of 73%. This indicates that there are variations between the isolates and extracts employed. The alcohol-free Api10 propolis extract employed in this study was observed to inhibit the mycelial growth of *F. proliferatum* by 70-100% at concentrations ranging from 0.925% to 1.125%. To the best of our knowledge, our study is the first to examine the effects of propolis extract on the mycelial growth rate of *F. proliferatum*.

The three concentrations of propolis extract, which inhibit mycelial growth above 70% (0.925%, 1.025%, 1.125%), were separately mixed with the conidia suspension of the pathogen and applied to the seeds of the pumpkin. Among the concentrations, 1.125% (Figure 4) was observed to have the lowest disease severity (42.70%) and to prevent disease severity by the highest rate (44.13%).

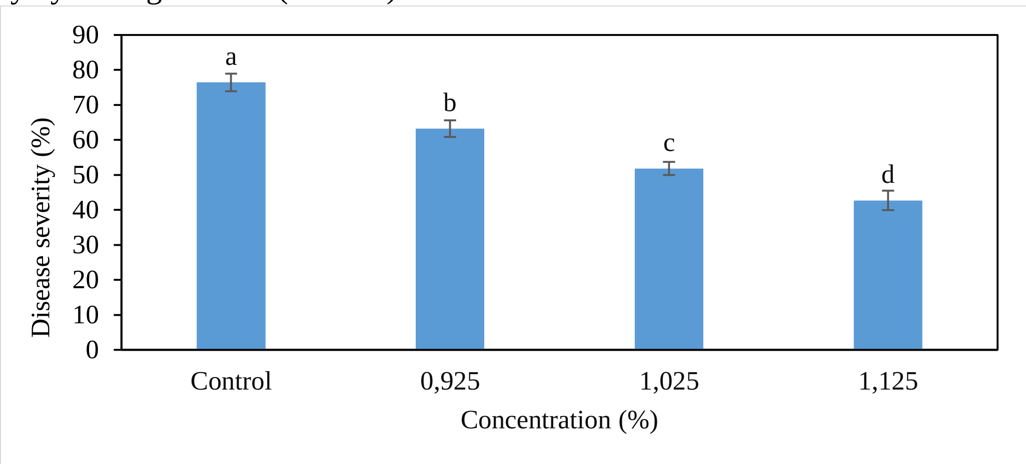


Figure 4. Disease severity of *Fusarium proliferatum* in different concentrations of propolis extract: Values are means ± standard error (SE) and bars shows SE. Bars topped by a different letter differ significanty based on the LSD test at P<0.005

The effects of various propolis extracts on diseases caused by *Colletotrichum gloeosporioides* in mango fruit (Mattiuz et al., 2015), *Monilia fructigena* in quince fruit (Özyiğit et al., 2018), *C. musea* in banana fruit (Dudoit et al., 2020), *Penicillum* spp., *Alternaria alternata* and *Botrytis cinerea* in raspberry fruit (Moredo et al., 2020) have been investigated. However, no study has yet investigated the potential of propolis extract to prevent the seedling rot caused by *F. proliferatum* in any plant species. In this context, the results of the present study on the effect of propolis extract against seedling rot caused by *F. proliferatum* in pumpkin represent the first findings.

CONCLUSION

Considering problems such as the increasing use of fungicides in recent years, environmental pollution and resistance of fungal species to fungicides, the present study includes an environmentally friendly application and is important in terms of sustainability in agriculture. It was determined that the propolis extract used in the current experiments inhibited mycelial development of *F. proliferatum* at increasing concentrations and had fungicidal activity against seedling root and crown rot caused by the pathogen. It also contributes significantly to the development of biocontrol strategies that can be applied under field conditions with seed coating. It also provides a basis for testing the efficacy of propolis in various agricultural crops other than pumpkin. It is expected that the results obtained will provide important contributions to the pot and field trials to be conducted later.

ACKNOWLEDGEMENTS

We would like to express our gratitude to Prof. Dr. İbrahim PALABIYIK for kindly providing samples of his lactic acid-based propolis (Api10) extract (Patent No: 2020/05364) for the purposes of this study. Furthermore, the authors would like to express their gratitude to AYFA Agriculture for providing the TG38 cultivar used in this study.

REFERENCES

Abdalla, M. Y., A. Al-Rokibah, A. Moretti, G. Mule. 2000. Pathogenicity of toxigenic *Fusarium proliferatum* from date palm in Saudi Arabia. Plant Disease, 84(3): 321-324.

Araújo, M. J. A. M., S. D. M. G. Bosco, J. M. Sforcin. 2016. *Pythium insidiosum*: inhibitory effects of propolis and geopropolis on hyphal growth. Brazilian Journal of Microbiology, 47: 863-869.

Bankova, V. S., S. L. de Castro, M. C. Marcucci. 2000. Propolis: recent advances in chemistry and plant origin. Apidologie, 31(1): 3-15.

Bayram, N. E., Y. C. Gerçek, G. C. Öz. 2020. Screening for antioxidant capacity, pollen types and phytochemical profile by GC/MS and UHPLC from propolis. Progress in Nutrition, 22: e2020011.

Borrero, C., N. Capote, M. A. Gallardo, M. Avilés. 2019. First report of vascular wilt caused by *Fusarium proliferatum* on strawberry in Spain. Plant Disease, *103*(3): 581-581.

Curifuta, M., J. V. Pardenilla, J. Sanchez, A. Contreras, L. A. Salazar, M. Alvear. 2012. The *in vitro* antifungal evaluation of a commercial extract of Chilean propolis against six fungi of agricultural importance. Ciencia e Investigación Agraria, 39(2): 347-359.

Çakar, G., I. S. Sivrikaya, E. Karakaya, A. Güller. 2022. Inhibition effect of different propolis extracts against *Fusarium solani in vitro*. Avrupa Bilim ve Teknoloji Dergisi, 35: 82-88.

Deans, S. G., K. P. Svoboda. 1990. The antimicrobial properties of marjoram (*Origanum majorana* L.) volatile oil. Flavour and Fragrance Journal, 5(3): 187-190.

Delen, N., M. Yıldız, M., H. Maraite. (1984). Benzimidazole and dithiocarbamate resistance of Botrytis cinerea on greenhouse crops in Turkey.

Demir, E., N. Özer, H. Bayraktar 2023. Identification of seed-borne fungi in summer (*Cucurbita pepo*) and winter (*Cucurbita moschata*) pumpkins of Turkey. Journal of Plant Pathology, 105(3): 1087-1101.

Desjardins, A. E., R. D. Plattner, P. E. Nelson 1997. Production of fumonisin B (inf1) and moniliformin by *Gibberella fujikuroi* from rice from various geographic areas. Applied and Environmental Microbiology, 63(5): 1838-1842.

Dudoit, A., C. Mertz, M. Chillet, N. Cardinault, P. Brat. 2020. Antifungal activity of Brazilian red propolis extract and isolation of bioactive fractions by thin-layer chromatography-bioautography. Food Chemistry, 327: 127060.

Easton-Calabria, A., K. C. Demary, N. J. Oner. 2019. Beyond pollination: honey bees (*Apis mellifera*) as zootherapy keystone species. Frontiers in Ecology and Evolution, 6: 161.

Escriche, I., M. Juan-Borrás. 2018. Standardizing the analysis of phenolic profile in propolis. Food Research International, 106: 834-841.

Ghaly, M. F., S. M. Ezzat, M. M. Sarhan. 1998. Use of propolis and ultragriseofulvin to inhibit aflatoxigenic fungi. Folia Microbiologica, 43: 156-160.

Ghedira, K., P. Goetz, R. Le Jeune. 2009. Propolis. Phytothérapie, 7(2): 100-105.

Haile, K., A. Dekebo. (2013). Chemical composition and antimicrobial activity of Haramaya propolis (bee glue), Ethiopia. International Journal of Pharmaceutical Sciences and Research, 4(2): 734.

Huang, S. C. P. Zhang, K. Wang, G. Q. Li, F. L. Hu. 2014. Recent advances in the chemical composition of propolis. Molecules, 19(12): 19610-19632.

Karman, M. 1971. Bitki Koruma Araştırmalarında Genel Bilgiler. Denemelerin Kuruluşu ve Değerlendirme Esasları. Gıda Tarım ve Hayvancılık Bakanlığı, Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü Yayınları, Mesleki Kitaplar Serisi, 278 s.

Logrieco, A., A. Moretti, A. Ritieni, A. Bottalico, P. Corda. 1995. Occurrence and toxigenicity of *Fusarium proliferatum* from preharvest maize ear rot, and associated mycotoxins, in Italy. Plant Disease, 79(7): 727-731.

Marcucci, M. C. (1995). Propolis: chemical composition, biological properties and therapeutic activity. Apidologie, 26(2): 83-99.

Mattiuz, B. H., M. N. Ducamp-Collin, C. F. M. Mattiuz, C. Vigneault, K. M. Marques, W. D. Sagoua, Montet. 2015. Effect of propolis on postharvest control of anthracnose and quality parameters of ‘Kent’mango. Scientia Horticulturae, 184: 160-168.

Meneses, E. A., D. L. Durango, C. M. García. 2009. Antifungal activity against postharvest fungi by extracts from Colombian propolis. Química Nova, 32: 2011-2017.

Mohammadzadeh, S., M. Shariatpanahi, M. Hamedi, R. Ahmadkhaniha, N. Samadi, S. N. Ostad. 2007. Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis. Food Chemistry, 103(4): 1097-1103.

Moreno, M. A., A. M. Vallejo, A. R. Ballester, C. Zampini, M. I. Isla, A. López-Rubio, M. J. Fabra. 2020. Antifungal edible coatings containing Argentinian propolis extract and their application in raspberries. Food Hydrocolloids, 107: 105973.

Ouahab, A., N. Grara, A. Debbi. 2023. Antifungal property of bee propolis collected from the province of Souk Ahras (northeast Algeria). Analele Universitãţii din Oradea, Fascicula Biologie, 30(1): 60-64.

Özyiğit, Ç., Y. Yanar, D. Yanar, A. Onaran. 2018. Ayvada kahverengi çürüklük (*Monilinia fructigena* Honey in Whetzel) hastalığının propolis etanol ekstraktı ile kontrolü. Journal of Agricultural Faculty of Gaziosmanpaşa University (JAFAG), 35(Ek Sayı): 108-113.

Pérez, B. A., M. F. Berretta, E. Carrión, E. R. Wright. 2011. First report of root rot caused by *Fusarium proliferatum* on blueberry in Argentina. Plant Disease, 95(11): 1478-1478.

Quiroga, E. N., D. A. Sampietro, J. R. Soberon, M. A. Sgariglia, M. A. Vattuone. 2006. Propolis from the northwest of Argentina as a source of antifungal principles. Journal of Applied Microbiology, 101(1): 103-110.

Ren, J., G. Zhang, Y. Y. Zhang, J. Zhang, H. L. Zheng, L. Jing, H. Y. Zhou, J. Zhao. 2015. First report of sunflower wilt caused by *Fusarium proliferatum* in Inner Mongolia, China. Plant Disease, 99(9): 1275-1275.

Ristivojević, P., T. Stević, M. Starović, S. Pavlović, M. M. Özcan, T. Berić, I. Dimkić. 2020. Phenolic composition and biological activities of geographically different type of propolis and black cottonwood resins against oral streptococci, vaginal microbiota and phytopathogenic *Fusarium* species. Journal Of Applied Microbiology, 129(2): 296-310.

Salatino, A., M. L. F. Salatino. 2021. Scientific note: Often quoted, but not factual data about propolis composition. Apidologie, 52: 312-314.

Sevinç, E., N. Özer. 2023. The Effects of Seed Infestation by *Fusarium proliferatum* on Root and Crown Rot, Plant Growth and Phenolic Compounds in Roots of Some Pumpkin (*Cucurbita pepo* L.) Cultivars. Turkish Journal of Agriculture-Food Science and Technology, 11(12): 2308-2313.

Toreti, V. C., H. H. Sato, G. M. Pastore, Y. K. Park. 2013. Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evidence-Based Complementary and Alternative Medicine, 2013(1): 697390.

Tosi, E. A., M. C. Ciappini, A. F. Cazzolli, L. M. Tapiz. 2006. Physico chemical characteristics of propolis collected in Santa Fe (Argentine). Apiacta, 41: 110-120.

Townsend, G. R., J W. Heuberger. 1943. Methods for estimating losses caused by diseases in fungicide experiments. Plant Disease Reporter, 27: 340-343

Yamazaki, M., Y. Morita, T. Kashiwa, T. Teraoka, T. Arie. 2013. *Fusarium proliferatum*, an additional bulb rot pathogen of Chinese chive. Journal Of General Plant Pathology, 79: 431-434.

Yang, S. Z., L. T. Peng, X. J. Su, F. Chen, Y. J. Cheng, G. Fan, S. Y. Pan. 2011. Bioassay-guided isolation and identification of antifungal components from propolis against *Penicillium italicum*. Food Chemistry, 127(1): 210-215.

Yu, X., J. Zhang, L. Guo, A. Yu, X. Wang, W. Xiang, J. Zhao. 2022. First Report of *Fusarium proliferatum* Causing Fruit Rot on Muskmelon (*Cucumis melo*) in China. Plant Disease, 106(4): 1305.

DETECTION OF SOME PLANT FUNGAL DISEASES BY IMAGE PROCESSING TECHNIQUE

Ömer Ümit OKÇU[1], Nuray ÖZER[1]

[1] Tekirdağ Namık Kemal University, Agricultural Faculty, Plant Protection Department, Tekirdağ, Türkiye

Corresponding author e-mail: ouokcu@nku.edu.tr

ABSTRACT

Image processing techniques, which are the subject of research in many fields in the world and in our country, have made great progress in recent years, especially in the field of agriculture. As the human population grows, so does the need for food. For this reason, it is particularly important to prevent fungal losses in crop production per unit area. Timely and proper control minimizes losses due to fungal diseases. Thanks to the developing technology, diseases can be easily identified with image processing techniques and a strategy to combat them can be determined. Plant fungal diseases can be detected quickly, accurately and conveniently using machine vision technology. By increasing the number of these identification studies and integrating them with drone technology, it will be possible to provide sustainable control of plant fungal diseases by saving both time and labor over very large areas. In this study, the history, importance, stages of application of image processing and some studies on its use in the detection of plant fungal diseases were mentioned.

Keywords: Agriculture 4.0, Smart agriculture, Drone technology, Artificial intelligence.

INTRODUCTION

In crop production, many factors, especially diseases, affect product quality and quantity. In addition, in crop production, inaccurate adjustment of practices and inputs, such as the use of diesel oil, chemicals, irregular irrigation, and failure to properly set the harvest date, may result in failure to obtain the desired product. With the introduction of modern technology in agriculture and the availability of unmanned aerial vehicles, the trend towards image processing techniques has also increased. It has become imperative to integrate agricultural production with technology. Agricultural production is almost as old as humanity (Teke et al., 2016). With the use of precision agriculture applications along with unmanned aerial vehicles and image processing facilities, studies on plant disease detection have also gained significant progress (Altaş et al., 2023; Matese et al., 2015; Zhang et al., 2002

Invisible wavelengths of light have many applications, particularly in the highly accurate detection of early-stage disease in crops (Chen et al., 1989; Chen et al., 2010). In today's world, where sustainable and good agricultural practices are gaining importance day by day, image processing techniques are used in areas such as leaf area determination, fruit maturity determination, plant disease diagnosis and differentiation, and disease severity calculation due to the integration of information technologies with agriculture (Karabacak, 2007; Mustafa et al., 2008; Örge, 2012; Sabancı and Aydın, 2014; Zhao et al., 2009).

In this review, the importance of image processing technology in the historical development of agriculture, the history of image processing, methods and steps, the importance of image processing in the detection of plant fungal diseases were emphasized and examples of some studies in this field were given.

HISTORY OF IMAGE PROCESSING

Remote sensing, which began in the 1800s, began with the first photograph taken by Joseph Nicephoce Niepce (Figure 1.A) and Gaspard Tournachon, who photographed a village in Paris from a flying balloon (Figure 1.B). In the 1860s, Professor Sam King and James Wallace Black took aerial photographs of the city of Boston from a balloon at an altitude of 1200 feet (Figure 1.C) (Baumann, 2001). To observe the course of the war from the air during the Civil War period in the United States, kites, pigeons (Figure 1.D) and various mechanisms were placed on weather balloons to obtain images (Colwell, 1983). The first aerial photograph was taken by Arthur Batut in 1989. He used a device attached to a kite. In 1903, pigeons were used for a similar process. During World War I, the photographic images were obtained through the use of airplane. (Figure 1.E). In the 2nd World War more advanced techniques were used. This was the first time that civilians had access to aerial photography (Lillesand and Kiefer, 2000). In 1929, a liquid-fueled rocket was launched into the air in the United States, and a camera and barometer were attached to (Figure 1.F) to take aerial photographs. The advent of the rocket, balloon, kite, pigeon and aeroplane marked a pivotal moment in history. The term 'remote sensing' was first coined in 1960, followed by the launch of the inaugural remote sensing satellite in 1972 (Bilgi, 2007).

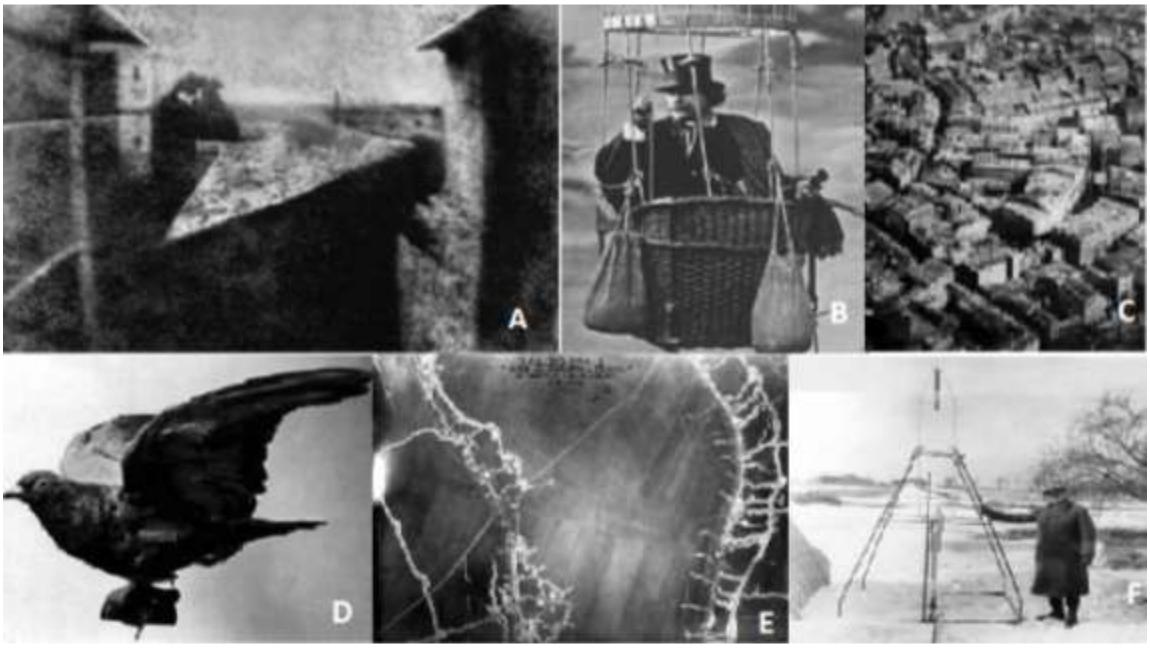


Figure 1. The first photograph taken by Joseph Nicephoce Niepce (A), the person who took the first aerial photograph in Paris (B), an aerial photograph of the city of Boston (C), a pigeon with a camera attached (D), an aerial photograph taken from an airplane during World War II (E), a camera attached to a rocket to take photographs (F) (Bilgi, 2007).

IMAGE PROCESSING METHODOLOGY

The main purpose of image processing is to identify and classify signs or objects that have common features or can be related to each other using previously identified features or characters (Demir et al., 2016). In light of the data obtained, it is possible to realize full-time and autonomous machines by bringing together various applications such as artificial intelligence, deep learning, modeling, machine learning, and simulation under a single roof. The digitization of the image is based on the process of converting the image in the camera into electrical signals with an optical-electrical mechanism (Solak and Altınışık, 2018). Image processing is employed in the field of agriculture for a multitude of purposes. These includes the detection of diseases, pests and weeds, the measurement of leaf area, the analysis of color, the classification of crops, the determination of yield, the creation of drought maps, the tracking of cattle and sheep, the assessment of soil fertility and moisture value (Dalen, 2004; Keefe, 1992).

IMAGE PROCESSING STEPS

The process of image processing, which can be divided into three fundamental stages, begins with the conversion of the original image into a digital format. This is followed by the editing of the digital image to achieve the desired format, and finally, the performance of the necessary analysis and evaluation of the results In these methods, the rays that identify the object or objects are converted into electrical signals in the camera device. In this manner, the image is converted to an analog format, which is a physical representation. In the final stage of the process, the image is converted into a digital format, enabling it to be processed within a computer environment. The aforementioned steps necessitate the utilisation of image sensors and systems that are capable of transforming the signals generated by the sensors into a digital format. In the event that the signals obtained from the sensors are in analog form, they are converted to digital format with the assistance of analog-to-digital converters. Analog signals are continuous signals that are functions of continuous variables, such as space, signal, or time. The value is generally constant. In the context of an analog image, regardless of the degree of magnification or the angle of observation, the colors that comprise the image remain unaltered. In order for a computer to process continuous functions or parameters, the functions must be digitized. A digital image is an analog image represented as a function of spatial coordinates (x, y) expressed in terms of discrete samples and represented as a function of spatial coordinates (x, y) (Karakoç, 2012).

IMAGE PROCESSING TECHNIQUES

The data obtained from the data libraries, created by obtaining thousands of images, are applied in three different ways. Firstly, in the context of the white-gray scale technique, it can be defined as a histogram representation of the white-gray balance. The objective is to reveal the shape in the image by utilising the white-grey scale in the obtained digital image. Given that each point in the image is distinct, the positioning of the bits required for each illumination level is also variable. The images of varying levels are expressed in gray-level or gray-level scale. The second technique is based on the identification of the digital image using the RGB values, which are the primary colors. All colors in the color spectrum can be derived from the combination of three primary colors: red (R), green (G), and blue (B). In this logic, referred to as the additive color space, the image is constructed from a combination of red, green, and blue light emitted by the pixels. Each pixel is assigned a reflectance or brightness value within the range of 0 to 255. If one of the red, green, or blue values is set to 255, while the others are set to 0, then one of the RGB values is present. In the event that all RGB values assume the value of 255, the resulting color is perceived as white. Conversely, if all RGB values assume the value of 0, the resulting color is perceived as black. The formation of other colors is a consequence of the combination of red, green, and blue (RGB) components within the range of values between 0 and 255. The thresholding process enables the removal of pixels with a specific value or outside the desired range. Finally, the obtained image is divided into squares, the desired region is selected and processing is performed in the selected region (Şin et al., 2019). Once the optimal technique and program have been selected for specific purposes and areas of use, the data must be processed, interpreted, and prepared for utilization. In order to facilitate the correct definition of data libraries, it is necessary to increase the number of images that are prepared for use. The data libraries facilitate the instantaneous reading, processing, interpretation, and detection of the image.

DISCRIMINATION OF HEALTHY AND SICK PLANTS USING IMAGE PROCESSING TECHNIQUE

All green, leafy plants engage in photosynthesis as a means of survival. Furthermore, the process of photosynthesis is influenced by external stress factors that are encountered throughout the various stages of plant development. The absorption of visible light by chlorophyll in plant leaves is a prerequisite for the continuation of photosynthesis. The cell structures present in the leaf enable it to reflect near-infrared light with great intensity. In the near infrared (NIR) spectrum, healthy, vibrant, and greener plants appear brighter than sick, lifeless, and spindly plants. In brief, the wavelength of light is also influenced by the health status of the plant (Teke et al., 2016). The Normalized Difference Vegetation Index (NDVI) is a metric used to analyze the life history of plants (Deng et al., 2018; Thenkabail et al., 2000; Wang et al., 2020). In the calculation of NDVI, the utilization of near infrared (NIR) and red (RED) light wavelength bands is employed, with the formula being calculated as NDVI= (NIR-RED) / (NIR+RED) (Tucker, 1979). The Normalized Different Vegetation Index (NDVI) assumes values within the range of -1 to +1. Figure 2 illustrates the vitality indicator scale. On this scale, a value of -1 to 0 indicates the objects or dead plants, a value of 0 to 0.33 indicates unhealthy plants, a value of 0.33 to 0.66 indicates moderately healthy plants, and finally a value of 0.66 to 1 indicates very healthy plants.

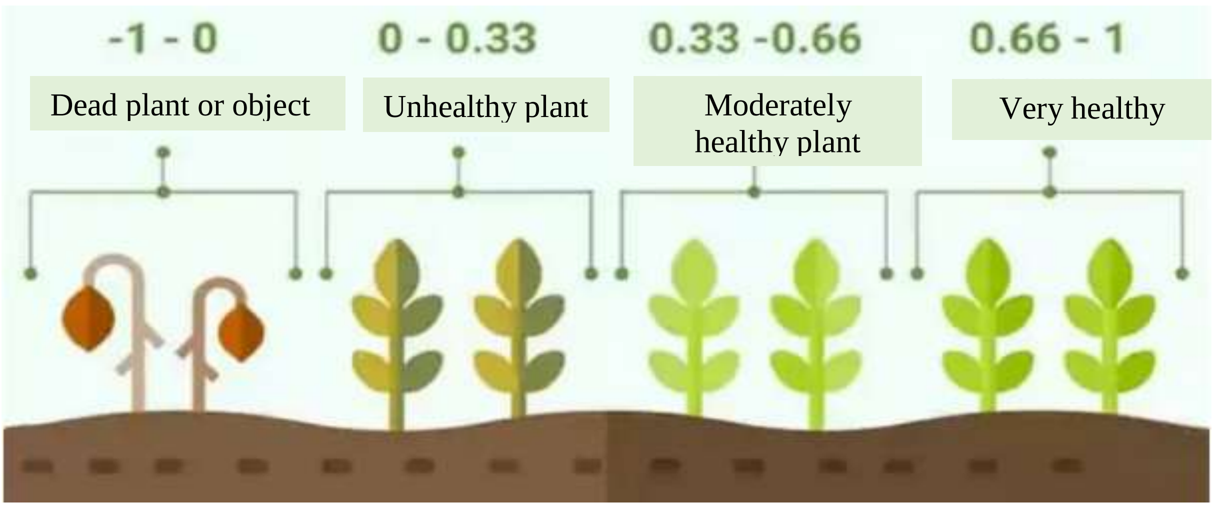


Figure 2. The plant vigor indicator scale (Anonymous, 2022)

STUDIES ON THE DETECTION OF FUNGAL DISEASES USING IMAGE PROCESSING TECHNIQUES

In the first study about the determination of fungal diseases with image processing techniques Wang et al. (2012) proposed a hybrid system based on image descriptors for the detection of four diseases such as grapevine downy mildew (*Plasmopara viticola* Berk. Et. Curt, Berl et de Toni), grapevine powdery mildew (*Uncinula necator* Schw. Burr.), wheat yellow rust (*Puccinia striiformis* f. sp. *tritici* West) and brown rust (*Puccinia recondita* f. sp. *tritici* Rob. Et Desm.). The diseased regions were delineated through the application of image segmentation, balancing, and k-means clustering algorithms. Subsequently, 50 feature vectors were derived through the utilisation of shape, colour, and texture-based methodologies. The efficacy of the aforementioned features, as determined through the utilisation of back propagation networks, was quantified. Consequently, the detection of vineyard and wheat diseases exhibited a 100% accuracy rate.

Quin et al. (2016), have selected the features using the methods of ReliefF, 1-Rule, Correlation-based feature selection (CFS) to define the symptoms of alfalfa leaf spot (*Pseudopeziza medicaginis* Libb, Sacc) (Figure 3A), alfalfa rust (*Uromyces striatus* J. Schroöt.) (Figure 3 C), Leptosphaerulina leaf spot (*Leptosphaerulina briosiana* (Pollacci) Graham et Luttrell) (Figure 3E) and Cercospora leaf spot disease (*Cercospora medicaginis* Ellis & Everh) (Figure 3G), the selected features were identified using ReliefF, 1-Rule, Correlation-based feature selection (CFS) methods. In the subsequent stage, the researchers created disease recognition models using random forest support vector machine (SVM), K nearest neighbour (KNN) supervised learning methods in accordance with the selected features. The results of this study indicated that the ReliefF method is the most effective for feature selection, while the SVM model is the optimal choice for the 45 features identified as most critical for recognition. The recognition accuracy of the training set and test set were calculated as 97.64% and 94.74%, respectively, for this model. The method enabled the generation of segmentation images of the symptoms of four alfalfa leaf diseases (Figure 3 B, D, F, H). It was suggested that the identification and recognition of the diseases exhibited a very high accuracy rate.

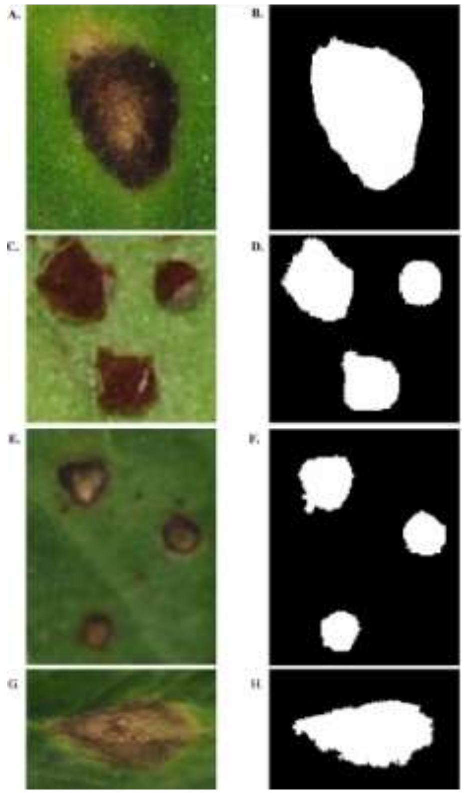


Figure 3. The symptoms (input) and segmentation (output) of some leaf diseases in alfalfa. A: Image of alfalfa leaf spot, B: Segmentation of alfalfa leaf spot, C: Image of alfalfa rust, D: Segmentation of alfalfa rust, E: Image of leptosphaerulina leaf spot, F: Segmentation of leptosphaerulina leaf spot, G: Image of cercospora leaf spot, H: Segmentation of cercospora leaf spot (Quin et al., 2016)

Sladojevic et al. (2016) identified 13 different foliar diseases, including powdery mildew (*Uncinula necator* Schw., Burr.), which is common in vineyard areas. In the study, more than 30,000 samples were used for convolutional neural networks. The results of the experimental studies indicated an accuracy rate of 91.11%. In the following year, Singh and Misra (2017) used genetic algorithm for image segmentation, which is an important feature in automatic detection and classification of plant leaf diseases. By combining image processing with different techniques, the presence of leaf spots was revealed as a result of segmentation of the image of the symptom caused by *Alternaria* spp. on bean leaves (Figure 4A) according to colour characteristics (Figure 4B). This approach was employed in order to identify plant diseases at an early or initial stage. Ramcharan et al. (2017), used a transfer learning approach based on deep convolutional neural networks for the detection of three disease agents in cassava plants. One of the diseases that can affect plants is brown leaf spot disease, which is caused by fungi. Using the pre-programmed Inception-v3 model, features were identified from diseased plant samples, the performance of these features was calculated using SVM and k-ECK classifiers, and as a result of experimental studies, 93% accuracy was obtained with the proposed approach and SVM classifier.

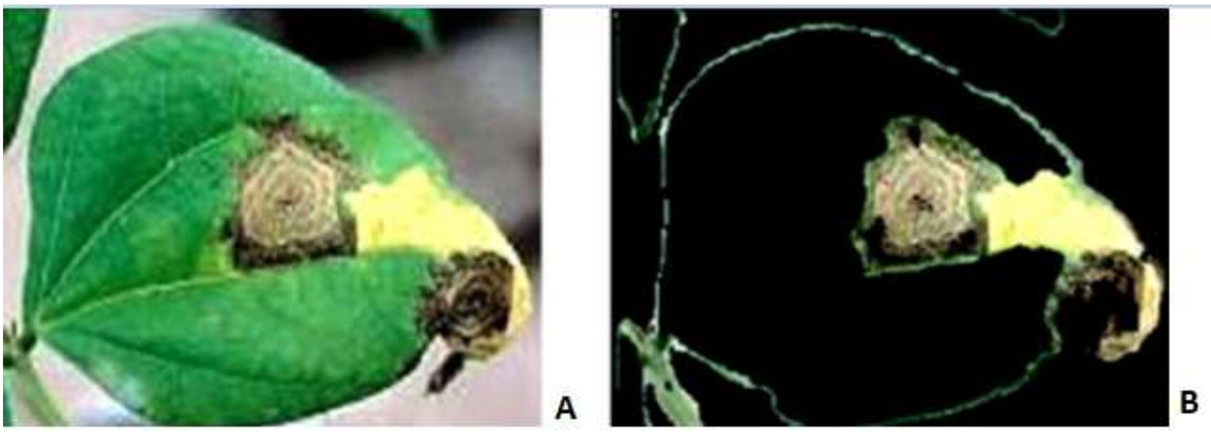


Figure 4. Leaf spot caused by *Alternaria* spp. on a bean leaf (A), and the leaf segmentation that was performed on the same leaf (Singh and Misra , 2017).

Türkoğlu and Hanbay (2018) used deep learning models such as Vgg 16, Vgg19 and AlexNet based on programmed convolutional neural networks (CNN) for the detection of freckle disease (*Wilsonomyces carpophilus* (Lev.) M.B. Ellis) in apricot and classified the deep features obtained from these models using the K-Nearest Neighbour (KNN) method. In addition, the dataset was contained a total of 960 images consisting of images of healthy and diseased apricots. As a result of the results obtained, the highest level of accuracy was obtained with the Vgg16 model, at 94.8%. In the same year, Walleling et al. (2018) proposed a new model based on deep neural networks for the classification and detection of brown spot (*Septoria glycines* Hemmi) disease in soybean. For the classification of the disease, the LeNet model was used. The PlantVillage database contained 12,673 leaf images, including four classes of healthy leaf images. As a result, the accuracy of the model used was 99.32%. This model was found to be able to extract important features and classify plant diseases from the captured images. Zhang et al. (2018) visually assessed the symptoms of leaf spot and mammalian rust diseases caused by *Alternaria* spp. (Figure 5 A) and *Gymnosporangium* spp. (Figure 5 B) in apple, respectively, and determined that incidence of disease was at the rate of 85.64%. KMSNN, SIFT and IRT image processing methods were then applied using the segmentation results (Figure 5 E, F) and disease detection rates of 75.87, 80.63 and 80.82% were achieved respectively. For *Colletotrichum orbiculare* (Berk. & Mont) Arx, (Figure 5 C) and *Erysiphe cichoracearum* (DC.) (Figure 5 D) in cucumber, the same methods were applied using the segmentation data (Figure 5 G,H) and while the disease detection rate obtained by the observer was 87.55%, this rate was 79.65% with KMSNN, 82.98% with SIFT and 84.08% with IRT. Through different methods, a new diseased leaf segmentation algorithm is proposed by integrating super pixel clustering and K-means clustering, which greatly reduces the computational cost.

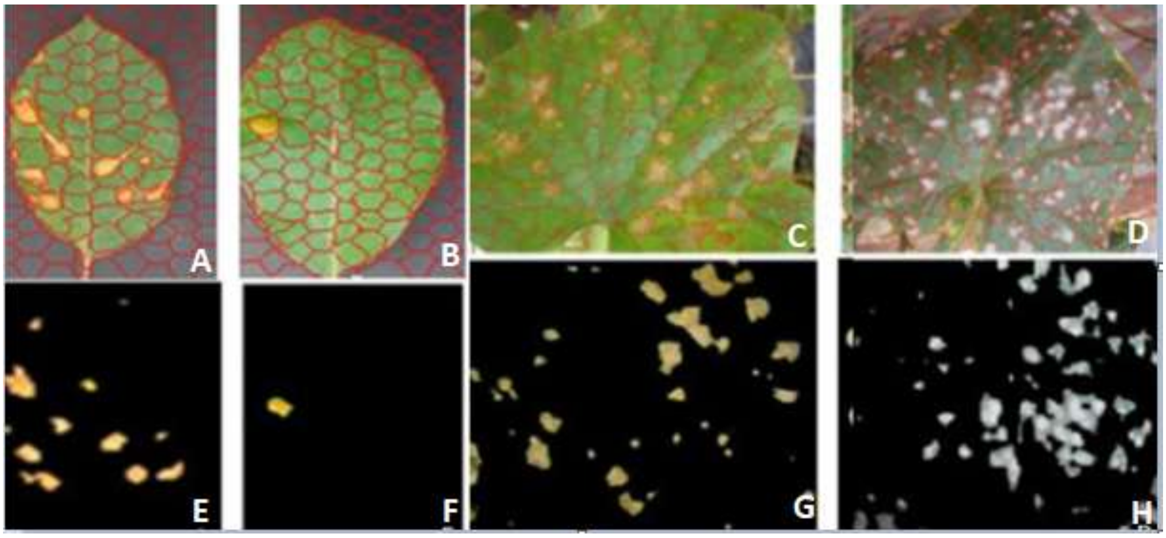


Figure 5. Images and segmentation of different fungal pathogens on the leaves. A: *Alternaria* and E: segmentation on apple leaf; B: *Gymnosporangium* spp and F: segmentation on apple; C: *Colletotrichum orbiculare* (Berk. & Mont) Arx, and G: segmentation on cucumber; D: *Erysiphe cichoreacearum* (DC.) and H: segmentation on cucumber (Zhang et al., 2018)

Altaş et al. (2019) determined sugar beet leaf spot (*Cercospora beticola* Sacc.) disease using segmentation process (Figure 6 A, B, C). They stated that a value of 48% was calculated with the image processing method on a leaf where 50% disease severity was calculated with the expert assessment result, and this value was very close to the expert assessment. The researchers used an image-processing algorithm they developed to calculate the exact location of the diseased areas. As a result of the study, it was emphasised that the imaging method can be preferred by obtaining precise values rather than observing. Özgüven and Adem (2019) proposed an updated, faster R-CNN model developed by changing the parameters of a CNN model for automatic detection of the same disease (Figure 7). When the proposed method was programmed with 155 images, the accuracy of the tested method was found to be 95.48% and it was reported that the proposed approach gave better results than the modern methods in the literature. Liang et al. (2019) used a convolutional neural network (CNN) model for disease detection of paddy leaf blight (*Pyricularia oryzae* Cav.). A data set with a total of 5808 samples has been designed for the testing and training of the model. As a result of the qualitative and quantitative experiments carried out to determine the effectiveness of the model, it was found to be the best performer. Zhang et al. (2019) developed a new three-channel ESA model for the classification of some problematic diseases, such as powdery mildew of cucumber (*Erysiphe cichoracearum* (DC.)) and Septoria leaf spot of tomato (*Septoria lycopersici* Speg.) (Figure 8 A, B, C, D). In this model, the proposed model is applied to each channel (R,G,B) of diseased plant leaf images and the learned weights obtained with the fully connected fusion layer are determined. To test the model, 15,817 coloured images of cucumber and tomato leaves were used. The images had 8 classes. As a result, it is confirmed that the proposed model has a better performance compared to the most recently developed methods.

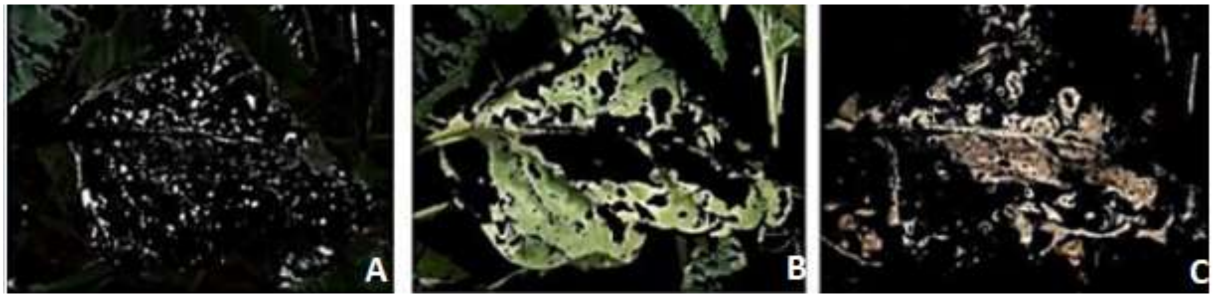


Figure 6. Leaf segmentation stages (A, B and C) according to colour characteristics of sugar beet leaf spot (*Cercospora beticola* Sacc.) disease (Altaş et al., 2019).

Figure 7. Testing process against sugar beet leaf spot (*Cercospora beticola* Sacc.) disease in sugar beet field using the proposed R-CNN model (Özgüven and Adem, 2019).

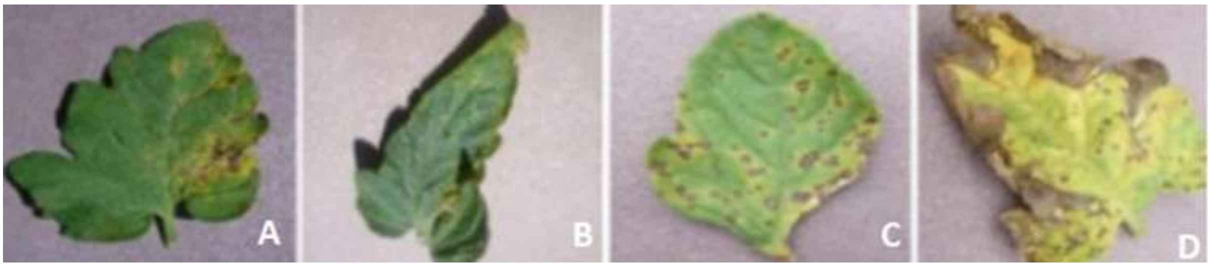


Figure 8. Different leaf samples (A, B, C, D) infected with Septoria leaf spot (*Septoria lycopersici* Speg.) in tomato (Zhang et al. 2019).

Aksoy et al. (2020) used convolutional neural network (CNN) models for detection of rust (*Gymnosporangium* spp.), black spot (*Venturia inaequalis* (CKe) Wint.) and black rot (*Alternaria* spp.) in apple with artificial intelligence. In this study, the best F-score, sensitivity, accuracy and specificity values of DenseNet-1, AlexNet, ResNet-34, Squeezenet1_0 and VGG16-BN models in CNN were determined by evaluating their performance according to the confusion matrix. As a result of the study, it was reported that the F-score, sensitivity, accuracy and specificity values were 98.62%, 97.64%, 99.52% and 99.54% respectively, and the best model was ResNet-34. Aslan (2021) tested a dataset of 327 colour images of monilia disease (Figure 9) in peach trees with the ESA AlexNet model and stated that the detection was made with an accuracy rate of 99.30% as a result of experimental studies.

Figure 9. Monilia disease on peach (Aslan, 2021).

Göksu et al. (2021) developed two models, namely EfficientNetB5 network and convolutional neural networks for the detection of corn rust (*Puccinia sorghi* Schwein) (Figure 10 A), northern (*Exserohilum turcicum* (Pass.) K.J. Leonard & Suggs) (Figure 10 B) and southern (*Bipolaris maydis* (Nisk. Miyale)) (Figure 10 C) leaf blight. The number of images in the performance matrices of the models has been increased using data multiplication techniques. As a result, the prediction rates obtained from the EfficientNetB5 transfer learning model and the developed deep learning model were 92.12% and 89.88% respectively.

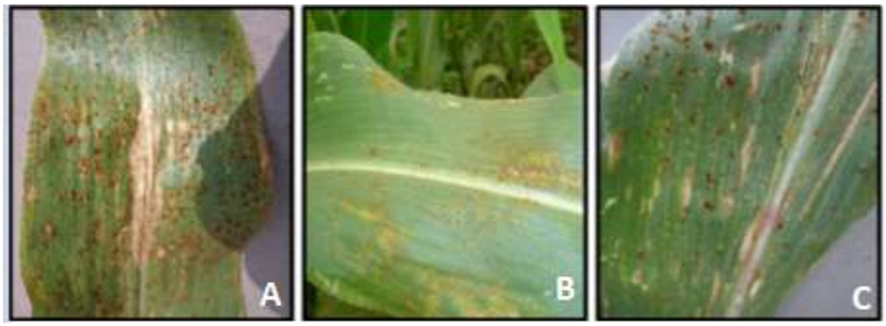


Figure 10. Some leaf diseases on the leaves of of maize. A: Rust (*Puccinia sorghi* Schwein); B: Northern (*Exserohilum turcicum* (Pass.) K.J.) leaf blight; C: Southern (*Bipolaris maydis* (Nisk. Miyale)) leaf blight (Göksu et al., 2021).

Atik (2022) used ResNet-18, ShuffleNet, SqueezeNet, AlexNet and GoogleNet models among the deep convolutional neural network (ESN) models for the detection of early leaf blight (*Alternaria solani* Sorauer), late leaf blight (*Phytophtora infestans* (Mont) de Bary) and Septoria leaf spot (*Septoria lycopersici* Speg) on tomato. The data set in the analyses is divided into 15% test data, 15% validation and 70% training. The accuracy rates of the models were 81.79%, 94.82%, 94.29%, 93.93% and 95.18% for ResNet-18, ShuffleNet, SqueezeNet, AlexNet and GoogleNet respectively. As a result of the analyses, GoogleNet was found to be the best performing of the pre-trained networks. Soydan and Taner (2022) used a total of 250 images, both ground and aerial for the identification of rice blight (*Pyricularia oryzae* Cav.) (Figure 11) using image processing techniques. The Levenberg-Marquardt training algorithm was used to train the neural networks, and the MCITG and MCRITD models were preferred to classify. The success rates of the MCITG and MCRITD models were 92.2% and 100% respectively. As a result, the applicability and effectiveness of the proposed method were determined. Ökten and Yüzgeç (2022) used a convolutional neural network (DNN), one of the machine learning methods for disease detection, to check for disease in rice plants. 5000 data sets of the leaves of the paddy plant were obtained from the Kaggle website. A total of two classifications were used for disease detection: infected plants with brown spot or leaf blight of rice and healthy plants. By modifying the hyperparameters of the ESA for disease detection, they achieved a success rate of 91.54%. The researchers obtained a total of 8000 images from the images in the dataset by means of a data augmentation method. After training on images for ESA, the success rate increased to 94.87%, and after training with ESA by preprocessing on the images in the dataset, this rate increased to 97.57%.

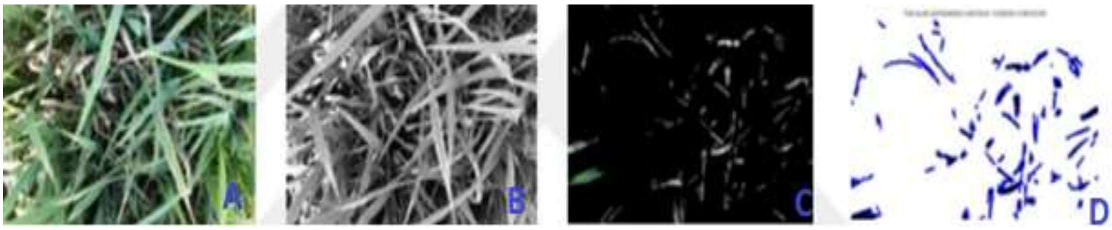


Figure 11. Disease symptom on the leaves (A) and segmentation according to colour characteristics (B, C, D) of rice blight caused by *Pyricularia oryzae* (Soydan and Taner, 2022).

Altaş et al., (2023), used faster R-CNN Model, which is an artificial intelligence approach, to detect and categorise some fungal diseases in vineyard, such as powdery mildew (*Uncinula necator* Schw, Burr.) (Figure 12 B), downy mildew (*Plasmopara viticola* Berk. Et. Curt, Berl et de Toni) (Figure 12 C) and Phomospis cane and leaf spot (*Phomopsis viticola* Sacc.) (Figure 12 D) in grapevine. They programmed with a total of 11000 images and compared them with healthy plants(Figure 12 A). As a result of the study, the accuracy rates of the diseases were calculated as: powdery mildew 95.83%, powdery mildew 96.61%, dead arm 97.12%. It is found that the proposed approach gives better results than other methods in the literature.

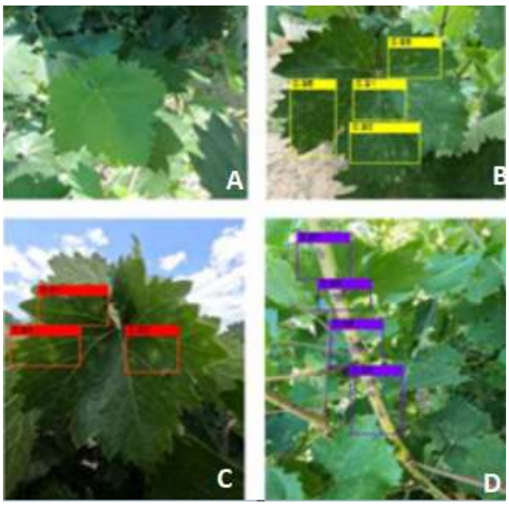


Figure 12. The process of applying the deep learning model A: healthy plant, B: powdery mildew (*Uncinula necator* Schw., Burr.), C: mildew (*Plasmopara viticola*

Berk. Et. Curt, Berl et de Toni), D: Phomospis cane and leaf spot (*Phomopsis viticola* Sacc.) (Altaş et al., 2023).

CONCLUSIONS

A review of previous studies shows that image processing techniques have been applied to many diseases, pests and weeds in the context of crop protection. However, in some cases, symptoms caused by abiotic or biotic factors may be confused and laboratory tests are required to make a definitive diagnosis. This highlights the importance of multidisciplinary studies. It is believed that researchers using imaging techniques will improve the quality and accuracy of their studies using the opinions of experts in the field of agriculture. Photographs taken in the field have been used with great success to identify plant samples under controlled conditions. However, when used in open terrain, success rates drop, so it is thought that the accuracy of the studies will increase as more use is made of the developing drone technology. To facilitate future studies, the number of data libraries on plant fungal diseases should be increased and studies should be given all kinds of support and assistance to keep up with the technological times in which we live.

REFERENCES

Aksoy, B., H. D. Halis, O. K. M. Salman. (2020). Detection of diseases in apple plants using artificial intelligence methods and comparison of the performance of artificial intelligence methods. International Journal of Engineering and Innovative Research, 2(3): 194-210.

Altas, Z., M. M. Özgüven, K. Adem. 2023. Automatic detection and classification of some vineyard diseases with Faster R-CNN model. Turkish Journal of Agriculture-Food Science and Technology, 11(1): 97-103.

Altaş, Z., M. M. Özgüven, Y. Yanar. 2019. The use of image processing techniques in determining plant disease and pest levels: sugar beet leaf spot disease example. International Erciyes Agriculture, Animal & Food Sciences Conferences. 24-27 April 2019- Erciyes University- Kayseri/Turkey.

Anonymous, 2022. Access Address: https://esular.com/ndvi-normallestirilmis-fark-bitki-ortusu-indeksi-nedir Access Date: 25.04.2024.

Aslan, M. 2021. Peach disease detection with deep learning. European Journal of Science and Technology, 23: 540-546.

Atik, İ. 2022. Performance analysis of plant leaf disease classification study with deep learning method. Kahramanmaraş Sütçü İmam University Journal of Engineering Sciences, 25(2): 126-137.

Baumann P. R. 2001. History of remote sensing aerial photogrammetry, Depatment of Geography State University of New York ABD.

Bilgi, S. 2007. Development and brief history of data acquisition methods in photogrammetry and remote sensing. Journal of Geodesy, Geoinformation and Land Methodology, 96: 48-55.

Chen X., Y. Xun, W. Li, J. Zhang. 2010. Combining discriminant analysis and neural networks for corn variety identification, Computers and Electronics in Agriculture, 71: 48-53.

Chen, P., M. J. McCarthy, R. Kauten. 1989 NMR for internal quality evaluation of fruits and vegetables, Trans. ASAE, 32: 1747–1753.

Colwell, R. N. 1983. Manual of remote sensing, American Society for Photogrammetry.

Dalen, G. V. 2004. Determination of the size distribution and percentage of broken kernels of rice using flatbed scanning and image analysis. Food Research International, 37: 51-58.

Demir, B., N. Çetin, Z. A. Kuş. 2016. Determination of colour characteristics of weeds using image processing technique. Alınteri Journal of Agricultural Sciences, 31(2): 59-64.

Deng, L., Z. Mao, X. Li, Z. Hu, F. Duan, Y. Yan. 2018. UAV-based multispectral remote sensing for precision agriculture: A comparison between different cameras. ISPRS Journal of Photogrammetry and Remote Sensing, 146: 124-136.

Göksu, M., K. M. Sünnetçi, A. Alkan. 2021. Disease detection in maize leaves using deep learning networks. Computer Science, (Special): 208-216.

Karabacak, H. 2007. Determination of Plant Surface Residue Coverage Rate with Image Processing Technique. Master's Thesis, Ankara University Institute of Science and Technology, Ankara.

Karakoç M. (2012). Image Processing Technologies and Applications. Master's thesis. Ege University Graduate School of Education. Department of Computer Engineering. Izmir 2012.

Keefe, P. D. 1992. A dedicated wheat grain image analyzer. Plant Varieties and Seeds, 5: 27-33.

Liang, W. J., H. Zhang, G. F. Zhang, H. X. Cao. 2019. Rice blast disease recognition using a deep convolutional neural network. Scientific Reports, 9(1): 2869.

Lillesand, T. R. Kiefer. 2000. Remote Sensing and Image Interpretation, 4th edition (New York: Johy Wiley ve Sons). New York, Wiley.

Matese A., P. Toscano, S. F. Di Gennaro, L. Genesio, F. P. Vaccari, J. Primicerio, C. Belli, A. Zaldei, R. Bianconi, B. Gioli, 2015. Intercomparison of uav, aircraft and satellite remote sensing platforms for precision viticulture. Remote Sensing, 7(3): 2971-90.

Mustafa, N. B. A., N. A. Fuad, S. K. Ahmed, A. A. Z. Abidin, Z. Ali, W. B. Yit, Z. A. Sharrif. M. 2008. Image processing of an agriculture produce: Determination of size and ripeness of a banana. Proc of International Symposium on Information Technology (Vol. 1, pp. 1-7).

Özguven M.M. K. Adem, 2019. Automatic detection and classification of leaf spot disease in sugar beet using deep learning algorithms, Physica A: Statistical Mechanics and its Applications, 535: 122537.

Ökten, İ., U. Yüzgeç. (2022. Detection of paddy plant disease by convolutional neural network. Bitlis Eren University Journal of Science and Technology, 11(1): 203-217.

Örge, G. 2012. Determination of colour characteristics of different cultivated plants and weeds by image processing technique. Master's Thesis, Namık Kemal University Institute of Science and Technology, Tekirdağ.

Quin, F., D. Liu, B. Sun, L. Ruan, Z. Ma, H. Wang. (2016). Identification of alfalfa leaf diseases using image recognition technology. *PloS One*, *11*(12): 0168274.

Ramcharan, A., K. Baranowski, P. McCloskey, B. Ahmed, J. Legg, D. P. Hughes. 2017. Deep learning for image-based cassava disease detection. Frontiers in Plant Science, 8: 1852.

Sabanci, K. C. Aydin. 2014. Image processing based precision spraying robot. Journal of Agricultural Sciences, 20(4): 406-414.

Singh, V. A.K. Misra. 2017. Detection of plant leaf diseases using ımage segmentation and soft computing techniques. Information Processing In Agriculture, 4: 41–49.

Sladojevic, S., M. Arsenovic, A. Anderla, D. Culibrk, D. Stefanovic. 2016. Deep neural networks-based recognition of plant diseases by leaf image classification. Computational Inteligance and Neuroscience, 3289801.

Solak, S., U. Altinışık. 2018. Detection and classification of hazelnut fruit using image processing techniques and clustering methods. Sakarya University Journal of Science, 22(1): 56-65.

Soydan, O., A. Taner. 2022. Identification of rice leaf blight disease by using ımage processing techniques. Anadolu Tarım Bilimleri Dergisi, 37(2): 341-360.

Şin, B., İ. Kadıoğlu, T. Sarı. 2019. Detection and identification of weed by image processing techniques. *BIALIC*, 7-8 November 2019, 40, Lviv Ukraine.

Teke, M., S. Deveci, F. Öztoprak, M. Efendioğlu, R. Küpçü, C. Demirkesen, F. F. Şimşek, B. Bağcı, E. Uysal, U. Türker, E. Yıldırım, Bayramin, İ. Kalkan, C. Demirpolat. 2016. Akıllı Tarım Fizibilite Projesi: Hassas Tarım Uygulamaları İçin Havadan ve Yerden Veri Toplanması, İşlenmesi ve Aanalizi. 6. Uzaktan Aalgılama-CBS Sempozyumu (UZAL-CBS 2016), 5-7 Ekim 2016, Adana.

Thenkabail, P. S., R. B. Smith, E. De Pauw. 2000. Hyperspectral vegetation indices and their relationships with agricultural crop characteristics. Remote Sensing of Environment, 71(2): 158-182.

Tucker, C. 1979. Red and photographic infrared linear combination for monitoring vegetation. Remote sensing of Environment, 8: 127- 150.

Türkoğlu, M., D. Hanbay. 2018. Apricot disease identification based on attributes obtained from deep learning algorithms. In *2018 International Conference on Artificial Intelligence and Data Processing (IDAP)*(pp. 1-4). IEEE.

Wallelign S., M. Polceanu, C. Buche. 2018. Soybean plant disease ıdentification using convolutional neural network, In The Thirty-First International Flairs Conference, Florida, pp.1-6.

Wang, H., G. Li, G. Ma, X. Li. 2012. Image recognition of plant diseases based on backpropagation networks, In 2012 5th International Congress on Image and Signal Processing, IEEE, Chongqing, China, pp.894-900.

Wang, L., J. Jin, Z. Song, J. Wang, L. Zhang, T. U. Rehman, D. Ma, N. R. Carpenter, M. R. Tuinstra. 2020. LeafSpec: An accurate and portable hyperspectral corn leaf imager. Computers and Electronics in Agriculture, 169: 105209.

Zhang, N., M. Wang, N. Wang. 2002. Precision agriculture-A worldwide overview. Computers and Electronics in Agriculture, 36(1): 113-132.

Zhang, S., W. Huang, C. Zhang. 2019. Three-channel convolutional neural networks for vegetable leaf disease recognition. Cognitive Systems Research, 53: 31- 41.

Zhang, S., H. Wang, W. Huang, Z. You. 2018. Plant diseased leaf segmentation and recognition by fusion of superpixel, K-means and PHOG. Optik, 157: 866-872.

Zhao, Y., D. Wang, D. Qian. 2009. Machine vision based image analysis for the estimation of pear external quality. In Intelligent Computation Technology and Automation, ICICTA'09. Second International Conference, 1: 629-632.

THE USE OF ZEBRAFISH (*Danio rerio*) AS BIOMEDICAL MODELS

Serim Tuna KOC[1], Suleyman KOK[2]

[1] *Department of Biotechnology and Genetics, Institute of Science and Technology, Trakya University, Edirne, Turkey*
[2] *Trakya University, Engineering Faculty, Genetic and Bioengineering Department, Edirne, Turkey*
Corresponding author e-mail: koks@trakya.edu.tr

ABSTRACT

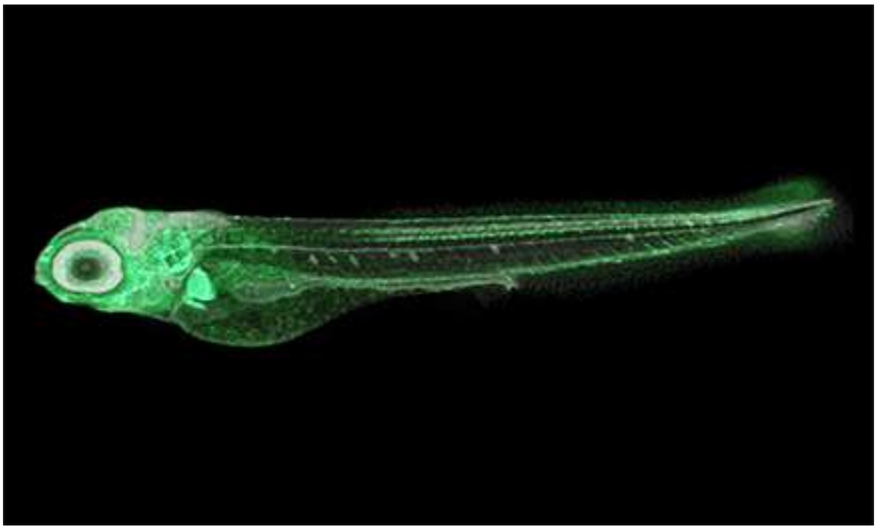
Various animal species have important roles as experimental models to advance biomedical research. Animal models play an important role in ensuring the consistency and validity of research results obtained from in vitro studies or studies with rodents. Today, zebrafish are considered a suitable model to investigate development, genetics, immunity, behaviour, physiology and nutrition. *Danio rerio,* the Latin name for zebrafish, formerly *Brachydanio rerio,* is a small tropical freshwater fish originating from the Ganges River and its tributaries in northern India. The annual number of publications on zebrafish as a model for biomedical research has increased significantly in recent years. The most advantageous features of zebrafish are its fully sequenced genome, easy manipulation of its genome, high fecundity, short generation time (about 3 months), rapid embryonic development (24 hours) and external fertilisation. The translucent zebrafish embryo allows the study of different developmental stages starting from the early stage of embryogenesis. Furthermore, zebrafish embryos form complete organ systems, including heart, intestine and blood vessels, within 48 hours after fertilisation. More than 10,000 mutants in protein-coding genes have been produced and several transgenic zebrafish lines have been constructed to study human diseases. The large number of zebrafish species available is another important advantage of this species. In addition, it is very economical to keep large numbers of zebrafish in a relatively small amount of laboratory space. In this review, information on the use of zebrafish as a bio-medical model, especially in areas related to diet-related diseases, metabolic disorders, liver diseases and intestinal diseases in humans, is compiled.

Keywords: Biomedical model, Metabolic disorders, Zebrafish

INTRODUCTION

For more than 200 years, fish have been used as model organisms by researchers. In this context, the oldest model species is the goldfish (Carassius auratus). Goldfish have primarily been used for applied studies in aquatic toxicology. However, other fish species such as zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), roach (*Rutilus rutilus*), three-spined stickleback (*Gasterosteus aculeatus*), pufferfish (*Takifugu rubripes*), and swordtail (*Xiphophorus hellerii*) have also been utilized in research (Ribas & Piferrer, 2014). Each fish species has its own unique advantages and disadvantages. For example, goldfish have been used in research on growth, stress, immunology, and reproduction. Medaka fish have become popular as a model species for genetic, reproductive, and developmental studies. In recent years, the use of zebrafish as a model organism has increased due to its suitable characteristics (Kutluyer and Aksakal, 2013).

The most advantageous features of zebrafish include: a fully sequenced genome, ease of genome manipulation, high fertility, short generation time (about 3 months), rapid embryonic development (24 hours), and external fertilization. The transparent zebrafish embryos allow for the study of different developmental stages from the early stages of embryogenesis (Figure 1). Additionally, zebrafish embryos develop complete organ systems, such as the heart, intestines, and blood vessels, within 48 hours of fertilization.



More than 10,000 mutants have been produced in protein-coding genes, and various transgenic zebrafish lines have been developed to study human diseases (Howe et al., 2013). The availability of numerous zebrafish strains and their economic maintenance in the laboratory are other significant advantages of this species. Despite its importance as a biomedical model, zebrafish have some limitations. For example, some organs, such as the respiratory and reproductive systems, do not completely overlap with human organs. Thus, using zebrafish as models for human respiratory or reproductive systems can be challenging. Additionally, because zebrafish live in an aquatic habitat, screening for some water-soluble drugs is another limitation (Song et al., 2016).

Various human diseases have been successfully modeled in zebrafish, including Duchenne muscular dystrophy, human melanoma, acute lymphoblastic leukemia, polycystic kidney disease, nephronophthisis, acute kidney disease, Parkinson's disease, Huntington's disease, Alzheimer's disease, myocardial infarction, and some metabolic disorders. This review focuses on common human metabolic diseases that have been successfully modeled in zebrafish, including obesity, type II diabetes mellitus, non-alcoholic steatohepatitis, and atherosclerosis (Gut et al., 2017).

General Characteristics of the Zebrafish

The scientific name of the zebrafish is *Danio rerio*, and it was previously known as *Brachydanio rerio*. This species is a small tropical freshwater fish native to the northern parts of the Ganges River and its tributaries in India (Tavares and Santos Lopes, 2013). In their natural habitats, zebrafish are typically found near the bottom of the water to minimize the risk of predation. The morphology of male and female zebrafish is illustrated in Figure

1.

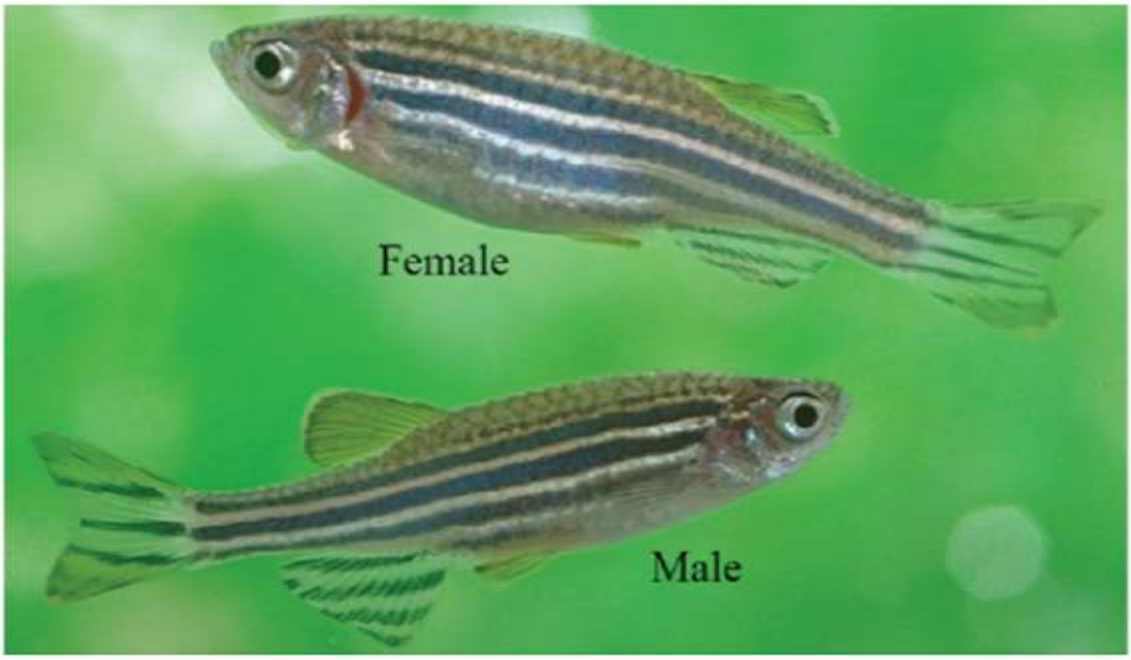


Figure 1. Adult male and female AB strain of zebrafish

Zebrafish Species and Research Usage

Despite the existence of numerous zebrafish species worldwide, the most commonly used varieties in biomedical research include AB, Casper, Ekkwill, Nadia, Wild Indian Karyotype, wild-caught, and Tubingen strains. According to the ZFIN website, over 800 biology laboratories around the world are conducting fundamental and applied research with zebrafish (https://zfin.org/search?q=Zebrafish+lab+oratories&category). In many of these laboratories, zebrafish are used to study human diseases such as neurological disorders, cancer, infectious diseases, cardiovascular diseases, kidney diseases, diabetes, blindness, deafness, digestive disorders, hematopoiesis, and muscle disorders (Figure 2).

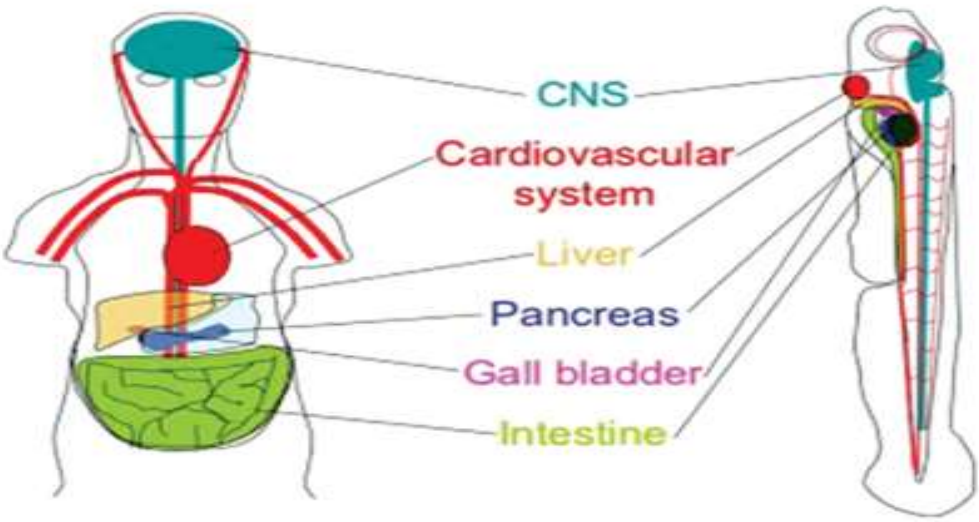


Figure 2. Some of the conserved organ systems between zebrafish and humans

Mutant zebrafish are created by knocking out specific genes, thereby generating new biomedical models. For instance, researchers interested in studying metabolic disorders can introduce different mutations in metabolism-related genes in zebrafish and track changes in gene expression using various molecular techniques. The short generation time of zebrafish complicates the production of stable transgenic adults or homozygous mutant embryos, often taking around 4 months. Recently, scientists have developed several technologies to accelerate the transgenic process (Burger et al., 2016). However, the presence or absence of genomic duplication events in zebrafish can complicate the study of certain human diseases.

Zebrafish as a Model for Metabolic Diseases

The disruption of the normal process of converting food into energy within cells can lead to various metabolic disorders. Although zebrafish (*Danio rerio*) have dietary requirements that differ from humans, they are considered a suitable model organism due to the conservation of many metabolic mechanisms. Various organs, such as the brain, intestines, liver, skeletal muscle, and adipose tissue, play roles in maintaining the balance between energy production and consumption. Zebrafish are particularly useful for studying metabolic dysfunctions due to their conserved lipid storage system and their ability to regulate appetite and insulin (Nishio et al., 2012).

According to the World Health Organization, cardiovascular diseases are currently among the leading fatal metabolic disorders (Lozano et al., 2012). Obesity (Ng et al., 2014), type 2 diabetes mellitus, and non-alcoholic fatty liver disease (LaBrecque et al., 2014) increase the risk of cardiovascular diseases. Zebrafish, with their similar metabolic organs such as digestive organs, adipose tissue, and muscles, are popular for studying metabolic disorders. Additionally, new tools and approaches, such as TALENs, CRISPR/Cas9 (Wu et al., 2018), compound therapies (Poureetezadi et al., 2016), mass spectrometry-based polar metabolomics and lipidomics (Zhang et al., 2018), and in vivo imaging with fluorescent dyes (Minchin et al., 2018), enable researchers to explore the molecular mechanisms of metabolic processes in zebrafish. Researchers have used zebrafish to study various metabolic diseases, including congenital metabolic errors, hyper and hypothyroidism, hypothalamic-pituitary-adrenal axis disorders, circadian rhythm disruptions, and cancer metabolism (Gut et al., 2017).

Zebrafish as a Model for Diet-Induced Obesity

The primary cause of diabetes mellitus is the inability of pancreatic β-cells to produce insulin, resulting in insulin deficiency. These functions and processes show similarities between zebrafish and humans. Zebrafish rapidly develop obesity and obesity-related diseases when exposed to hypercaloric and high-fat diets, activating metabolic pathways similar to those in humans. When glucose is present in the diet, the pancreas produces insulin, which inhibits gluconeogenesis through the downregulation of genes involved. In the absence of glucose in the bloodstream, gluconeogenesis is induced by glucagon. Capiotti et al. (2014) found a 41% increase in fructosamine (glycated protein) levels in the eyes of zebrafish immersed in a high-glucose solution (111 mM) for 14 days, along with decreased mRNA levels for insulin receptors in the muscles and the development of hyperglycemia. Zang et al. (2017) developed a zebrafish type 2 diabetes mellitus model by overfeeding with a high-calorie diet (408 calories per fish per day). Gene expression profiling in the liver and pancreas indicated a shared pathway for type 2 diabetes mellitus development between zebrafish and humans.

Connaughton et al. (2016) examined the relationship between age and type 2 diabetes mellitus and noted that younger zebrafish (4 to 11 months old) developed hyperglycemia more slowly compared to older zebrafish despite increased glucose concentrations. The glucose concentration in homeostatic organs can be increased by immersing zebrafish embryos in a glucose solution. Gleeson et al. (2007) demonstrated that immersing adult zebrafish in a 1% glucose solution for 24 hours raised blood glucose levels up to 400 mg/dL. Zang et al. (2017) developed two transgenic insulin resistance models, including one with dominant-negative IGF-I receptor expression in skeletal muscle and another with liver-specific knockout of the insulin receptor gene using CRISPR/Cas9 (Yin et al., 2015). These results highlighted zebrafish as a suitable model for studying glucose-related human diseases. Marín-Juez et al. (2014) developed a zebrafish model for hyperinsulinemia by injecting recombinant human insulin into zebrafish larvae. These studies revealed upregulation of the negative immune modulator protein tyrosine phosphatase non-receptor type 6 in insulin-resistant larvae. Yang et al. (2018) found that mutant zebrafish with insulin receptor gene knockouts fed a high-carbohydrate diet (%41) exhibited symptoms similar to human lipodystrophy, including hyperglycemia, decreased growth hormone signaling, increased visceral adiposity, and fatty liver development. Glucose levels in zebrafish can be measured using two types of glucose meters designed for diabetic patients (Eames et al., 2010). Additionally, fasting can be used for postprandial glucose and intraperitoneal glucose tolerance tests. Various methods, such as q-PCR (Michel et al., 2016), insulin antibody staining (Kimmel et al., 2015), or semi-quantitative dot-blot (Olsen et al., 2012), are available for measuring insulin levels in zebrafish. Insulin sensitivity can also be assessed by intraperitoneal insulin injection in hyperglycemic zebrafish (Capiotti et al., 2014).

Zebrafish as a Model for Dyslipidemia and Atherosclerosis Diseases

Increased levels of cholesterol, triglycerides, or high-density lipoprotein cholesterol lead to dyslipidemia, which can result in atherosclerosis. Given the well-known dietary requirements of zebrafish, many researchers have created various models by modifying standard diets (e.g., feeding zebrafish high-fat diets to induce obesity, hyperglycemia, and dyslipidemia). Zebrafish fed a high-cholesterol diet show histopathological changes similar to those observed in human atherosclerosis (Fang and Miller, 2012). The formulation of high-cholesterol diets plays a crucial role in dyslipidemia studies (Oka et al., 2010). Miyares et al. (2014) used the stages of yolk metabolism in zebrafish embryos to define lipid and lipoprotein metabolism and demonstrated that the inclusion of exogenous fatty acids in the circulatory system was dependent on lipoprotein production.

Zebrafish Model for Non-Alcoholic Fatty Liver Disease and Other Liver Disorders

Non-alcoholic fatty liver disease (NAFLD) is characterized by excessive fat accumulation in the liver and is not related to excessive alcohol consumption. This condition can lead to steatosis, steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. NAFLD is associated with insulin resistance, high-fat diets, drug-induced liver damage, and metabolic syndrome. Various studies have shown that zebrafish develop hepatic steatosis when exposed to hepatotoxic chemicals, starvation, or excessive dietary fat, cholesterol, or carbohydrates. These mechanisms are similar in zebrafish and humans. Interestingly, the first article on zebrafish development (Roosen, 1937) investigated the effects of different toxins, alcohol, and various levels of carbohydrate or fat diets on zebrafish embryos, larvae, and adult developmental stages. Applying toxins to fish tanks is a simple technique, making zebrafish a popular model for chemical screening mechanisms. The zebrafish liver closely resembles the human liver in cellular structure, function, and genetics. This observation has led researchers to use zebrafish for detailed embryological and genetic studies related to human liver development, as well as exploring potential treatments for liver disorders and diseases. Developing liver tumors in zebrafish using carcinogenic substances and comparing gene expression with human liver tumors emphasizes the significance of zebrafish as a biomedical model. Tonin et al. (2018) showed that zebrafish immersed in 6% fructose developed hepatic steatosis similar to symptoms seen in humans on a high-carbohydrate diet. Yang et al. (2019) demonstrated that overfeeding led to fatty liver development and accelerated carcinogenic processes. Additionally, the leptin hormone responsible for obesity was not regulated in oncogenic and overfed zebrafish. They found it possible to reduce muscle wasting phenotype by downregulating leptin signaling. The development of a mutated kazal liver gene in zebrafish has led scientists to investigate hepatic steatosis development and related molecular mechanisms. Furthermore, the development of gonzo mutant zebrafish has shown that sterol regulatory element-binding proteins mediate alcohol-induced hepatic steatosis (Passeri et al., 2009). Shimada et al. (2015) used a diet-induced obesity model in zebrafish liver to apply transcriptomic and proteomic methods to isolate genes responsible for hepatic steatosis. These studies revealed upregulation of fatty acid-binding protein 3 and transcription factors (E2F) in hepatic steatosis zebrafish. Howarth et al. (2013) developed two models using zebrafish to investigate steatosis caused by tunicamycin or ethanol, leading to liver failure. They prevented ethanol-induced steatosis by blocking the activation of sterol regulatory element-binding proteins using mutant zebrafish. These studies found hepatocyte dysfunction even without lipid accumulation. Imran et al. (2018) tested the role of membrane remodeling in hepatotoxicity using zebrafish larvae and found that exposure to benzo[a]pyrene and ethanol together caused in vivo hepatotoxicity through membrane remodeling. These findings have led scientists to develop treatments for non-alcoholic fatty liver disease and associated risk factors.

Zebrafish as a Model for the Study of Intestinal Diseases and Host-Microbe Interactions

The zebrafish intestine is a long, tube-like structure consisting of three main regions: the foregut, midgut, and hindgut, which folds twice in the abdominal cavity. The intestinal epithelium contains three different cell types: absorptive enterocytes, mucus-secreting goblet cells, and hormone-producing enteroendocrine cells. As zebrafish develop, the intestinal epithelium undergoes various changes, including differences in the composition of intestinal microflora (Sanders et al., 2019). The intestinal microbiota helps digest dietary components and contributes to maintaining homeostasis. Studying intestinal microbiota in zebrafish is a powerful tool for understanding the pathogenesis of intestinal diseases and host-microbe interactions. Additionally, zebrafish provide a suitable model for studying the effects of diet on the gut microbiome. By manipulating the diet of zebrafish, researchers can identify interactions between dietary factors, the gut microbiome, and the development of diseases such as irritable bowel syndrome, colitis, and colorectal cancer.

CONCLUSION

Thanks to its completely sequenced genome, high fertility, external fertilization, clarity of genetic manipulation, rapid development, and almost transparent embryos, the zebrafish is a superb model organism for biomedical research, including research into biological processes and diseases common to humans. Zebrafish have all major organs involved in the metabolism process and can be used to study various human metabolic disorders such as non-alcoholic fatty liver disease, type 2 diabetes, dyslipidaemia and other liver diseases. Zebrafish will continue to be an important biomedical model in the future with innovations and advances in molecular techniques.

REFERENCES

Bates, J. M., Akerlund, J., Mittge, E., Guillemin, K. 2007. Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. *Cell Host & Microbe, 2,* 371–382.

Fang, L., Miller, Y. I. 2012. Emerging applications for zebrafish as a model organism to study oxidative mechanisms and their roles in inflammation and vascular accumulation of oxidized lipids. Free Radical Biology and Medicine, 53, 1411–1420.

Gleeson, M., Connaughton, V., Arneson, L. S. 2007. Induction of hyperglycaemia in zebrafish (*Danio rerio*) leads to morphological changes in the retina. Acta Diabetologica, 44, 157–163.

Gut, P., Reischauer, S., Stainier, D. Y. R., Arnaout, R. 2017. Little fish, big data: zebrafish as a model for cardiovascular and metabolic disease. Physiological Reviews, 97, 889–938.

Howarth, D. L., Yin, C., Yeh, K., Sadler, K. C. 2013. Defining hepatic dysfunction parameters in two models of fatty liver disease in zebrafish larvae. Zebrafish, 10, 199–210.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., Collins, J. E., Humphray, S., McLaren, K., Matthews, L., et al. 2013. The zebrafish reference genome sequence and its relationship to the human genome. Nature, 496, 498–503.

Imran, M., Sergent, O., Tete, A., Gallais, I., Chevanne, M., Lagadic-Gossmann, D., Podechard, N. 2018. Membrane remodeling as a key player of the hepatotoxicity induced by co-exposure to benzo[a]pyrene and ethanol in obese zebrafish larvae. *Biomolecules, 8*(2), 26.

Ji, J., Thwaite, R., Roher, N. 2018. Oral intubation of adult zebrafish: A model for evaluating intestinal uptake of bioactive compounds. Journal of Visualized Experiments, 139, e58366.

Kutluyer, F., Aksakal, E. 2013. Sucul Model Organizmalar ve Biyoteknolojide Kullanımı. Anadolu Tarım Bilimleri Dergisi, 28(2), 101-107.

LaBrecque, D. R., Abbas, Z., Anania, F., Ferenci, P., Khan, A. G., Goh, K. L., Hamid, S. S., Isakov, V., Lizarzabal, M., Peñaranda, M. M., et al. 2014. World gastroenterology organisation global guidelines: Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Journal of Clinical Gastroenterology, 48(6), 467–473.

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S. Y., et al. 2012. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the global burden of disease study 2010. The Lancet, 380, 2095–2128.

Michel, M., Page-McCaw, P. S., Chen, W., Cone, R. D. 2016. Leptin signaling regulates glucose homeostasis, but not adipostasis, in the zebrafish. Proceedings of the National Academy of Sciences USA, 113, 3084–3089.

Minchin, J. E. N., Scahill, C. M., Staudt, N., Busch-Nentwich, E. M., Rawls, J. F. 2018. Deep phenotyping in zebrafish reveals genetic and diet-induced adiposity changes that may inform disease risk. Journal of Lipid Research, 59, 1536–1545.

Miyares, R. L., de Rezende, V. B., Farber, S. A. 2014. Zebrafish yolk lipid processing: A tractable tool for the study of vertebrate lipid transport and metabolism. Disease Models & Mechanisms, 7, 915–927.

Nishio, S., Gibert, Y., Berekelya, L., Bernard, L., Brunet, F., Guillot, E., Le Bail, J. C., Sánchez, J. A., Galzin, A. M., Triqueneaux, G., et al. 2012. Fasting induces CART down-regulation in the zebrafish nervous system in a cannabinoid receptor 1-dependent manner. Molecular Endocrinology, 26, 1316–1326.

Oehlers, S. H., Flores, M. V., Okuda, K. S., Hall, C. J., Crosier, K. E., Crosier, P. S. 2011. A chemical enterocolitis model in zebrafish larvae that is dependent on microbiota and responsive to pharmacological agents. Developmental Dynamics, 240, 288–298.

Olsen, A. S., Sarras, M. P., Jr., Leontovich, A., Intine, R. V. 2012. Heritable transmission of diabetic metabolic memory in zebrafish correlates with DNA hypomethylation and aberrant gene expression. Diabetes, 61, 485–491.

Passeri, M. J., Cinaroglu, A., Gao, C., Sadler, K. C. 2009. Hepatic steatosis in response to acute alcohol exposure in zebrafish requires sterol regulatory element binding protein activation. Hepatology, 49, 443–452.

Poureetezadi, S. J., Cheng, C. N., Chambers, J. M., Drummond, B. E., Wingert, R. A. 2016. Prostaglandin signaling regulates nephron segment patterning of renal progenitors during zebrafish kidney development. eLife, 5, e17551. https://doi.org/10.7554/eLife.17551

Ribas, L., Francesc, P. 2014. The zebrafish (*Danio rerio*) as a model organism, with emphasis on applications for finfish aquaculture research. Journal of Reviews on Aquaculture, 6 (4), 209–240.

Semova, I., Carten, J. D., Stombaugh, J., Mackey, L. C., Knight, R., Farber, S. A., Rawls, J. F. 2012. Microbiota regulate intestinal absorption and metabolism of fatty acids in the zebrafish. *Cell Host & Microbe*, *12*, 277–288.

Tavares, B., Santos Lopes, S. 2013. The importance of zebrafish in biomedical research. Acta Médica Portuguesa, 26 (5), 583–592. Retrieved from

Tonin, F. J., Raquel, A. O. H., da Silveira, T. R., Uribe-Cruz, C. 2018. Experimental model of hepatic steatosis by fructose in adult zebrafish: A pilot study. Clinical and Biomedical Research, 38(2), 151–154.

Yang, B. Y., Zhai, G., Gong, Y. L., Su, J. Z., Peng, X. Y., Shang, G. H., Han, D., Jin, J. Y., Liu, H. K., Du, Z. Y., et al. 2018. Different physiological roles of insulin receptors in mediating nutrient metabolism in zebrafish. American Journal of Physiology-Endocrinology and Metabolism, 315, E38–E51.

Yin, L., Maddison, L. A., Li, M., Kara, N., LaFave, M. C., Varshney, G. K., Burgess, S. M., & Patton, J. G. 2015. Multiplex conditional mutagenesis using transgenic expression of Cas9 and sgRNAs. Genetics, 200, 431–441.

Zang, L., Shimada, Y., Nishimura, N. 2017. Development of a novel zebrafish model for type 2 diabetes mellitus. Scientific Reports, 7, 1461.

OPTIMIZING FODDER PEA YIELD: IMPACT OF PLANTING DENSITY AND ROW SPACING

Adrian Maho[1], Jonida Biturku[2].*

[1] "Fan S.Noli" University, Faculty of Agriculture, Department of Agronomy, Korçe, Albania.

[2]Agricultural University of Tirana, Faculty of Agriculture and Environment, Tirana, Albania.

**Corresponding author: Adrian Maho E-mail: maho.adrian@yahoo.com*

ABSTRACT

The aim of the study was to evaluate the influence of the number of plants and the distance between the rows on the production of fodder pea. The experiment was conducted in 2020-2023. Fodder pea "Voskopoja" (landrace with the greatest spread in this area of Korçë district, Albania), was studied by applying three sowing densities (60, 80 and 100 seeds per 1 m^2), with three row spacings (20, 30 and 40 cm), in four replicates in 24 m^2 plots. The R factor, row spacing showed statistically significant influence on the number of seeds per pod, seed weight per pod and seed yield of fodder pea. Factor D planting density showed statistically significant effect on number of pods per plant, number of seeds per pod, seed weight per pod and seed yield of forage pea. The R x D interaction showed statistically significant effects on the number of seeds per pod, seed weight per pod and seed yield of forage pea. The number of plants and the distance between rows did not reflect significant changes in the weight of 1000 seeds. These factors did not reflect significant changes in protein content. This study showed that the interaction between the factors plant number and row spacing in winter pea modifies the yield of green mass, seeds, plant height and seed yield.

Keywords: Forage pea, Number of plants, Row spacing, Yield, Protein.

INTRODUCTION

Fodder pea "Voskopoja", named for its prevalence in the Voskopoja region, emerged from a selection of wild pea plants around 1980. This cultivar exhibits unique properties that make it particularly suitable for mountainous Mediterranean climates. Its most notable characteristic is its resistance to low temperatures, making it the only cultivar capable of autumn planting in these regions, particularly at altitudes exceeding 1200 meters. Farmers often cultivate "Voskopoja" alongside rye and potatoes, contributing to the overall sustainability of agricultural ecosystems in these challenging environments. (Mirzad et al., 2023)

Despite its resilience, "Voskopoja" faces challenges in maintaining consistent forage yields due to the fluctuating climatic conditions of mountainous Mediterranean regions. However, its ability to thrive in these harsh environments makes it an invaluable resource.

Leguminous crops like "Voskopoja" hold significant promise for diversifying food sources and enhancing the sustainability of agricultural systems, particularly in Europe. (Ditzler et al., 2021) No other annual legume demonstrates comparable resilience and adaptability to the ecological constraints faced by "Voskopoja".

The nutritional value of "Voskopoja" is substantial. As a forage crop, it serves as a valuable source of protein in livestock diets. Analyses reveal that "Voskopoja" contains approximately 25.04% crude protein and 48.54% starch. (Trupa et al., 2018) Further analysis of the grains indicates a composition of 84% dry matter, 26.8% protein, 2.3% fat, 14.1% cellulose, 48.7% non-nitrogenous extracts, and 8.1% ash. This rich nutritional profile underscores its significance as a high-quality feed source.

Beyond its use as green fodder, dry fodder, or fodder meal, "Voskopoja" seeds offer further versatility. Studies have demonstrated the positive effects of incorporating processed peas into dairy cow diets, highlighting their potential as a partial substitute for soy flour. (Volpelli et al., 2010)

The nitrogen-fixing capabilities of "Voskopoja" further enhance its value. Unlike other legumes, this cultivar does not form symbiotic relationships with nitrogen-fixing bacteria. Instead, it directly enriches the soil with approximately 80-100 kg/ha of biological nitrogen, acting as an excellent precursor for crops like wheat, barley, and rye. This natural fertilization process contributes to soil health and reduces reliance on synthetic nitrogen fertilizers. (Kumar and Goh, 2000)

The adaptability of "Voskopoja" extends to sloping terrains, where it maintains high productivity while mitigating erosion risks. This characteristic, coupled with its nitrogen-fixing abilities, positions it as a key player in promoting sustainable agricultural practices and reducing environmental impact. Moreover, its role in enhancing soil fertility through symbiotic nitrogen fixation significantly contributes to the sustainability of mountainous ecosystems, making it an exceptionally valuable crop for farmers in these regions. (Sallaku et al., 2016)

"Voskopoja" exhibits remarkable drought resistance, a crucial trait in rain-fed agricultural systems. (Kachout et al., 2021) Unlike other cultivars, its autumn planting allows for a period of intensive growth during the favorable temperatures of May and June.

Plant density is determined by the number of seeds planted per m^2. Plant density is one of the factors that affects the morphological and productive development of plants. From a biological point of view, the density of plants affects the competition between plants for lighting and food elements. Adequate plant density is also important against winter damage, especially for fall plantings. (Knott and Belcher, 1998)

The distance between rows is one of the important technical elements that affects the progress of plant development. The optimal distance between rows also varies between cultivars of the same species. It is conditioned by the different biological characteristics of the cultivars and by other agrotechnical factors. Cultivation of fodder pea "Voskopoja" in hilly lands and in the absence of irrigation is another element that shows influence. Therefore, it can only be determined experimentally.

Optimum density in fodder pea planting ensures better competitive ability with weeds by increasing interspecific competition.

Different plant density significantly modifies yield, plant height and number of pods/plant in forage pea. (Krizmanic et al., 2020), Yucel, 2013)

This study aimed to investigate how row spacing and planting density influence the yield of fodder pea "Voskopoja" in the Korçë district of Albania.

MATERIALS AND METHODS

Experimental Design and Site Description

This study, investigating the impact of row spacing and planting density on the yield of fodder pea "Voskopoja", was conducted over three consecutive years (2020-2023) in Trestenik, Devoll, Korçë, Albania (40°34'55"N; 21°01'36"E) at an altitude of 955 meters. The experiment was implemented on medium clay soil characterized by calculated fertility, utilizing a wheat-pea crop rotation system.

Fodder pea "Voskopoja" (landrace with the greatest spread in this area of Korçë district, Albania), was studied using a randomized complete block design with four replications. A total of nine treatment combinations were evaluated, encompassing three row spacings (20 cm, 30 cm, and 40 cm) and three planting densities (60, 80, and 100 seeds per m^2). Each plot measured 24 m^2 (2.4 m x 10 m), resulting in 12, 8, and 6 rows per plot for the respective row spacings. Seed quantities, based on a 1000-seed weight of 150g, were adjusted to 9g, 12g, and 15g per m2 for the corresponding planting densities. Planting was consistently carried out between September 20th and 30th throughout the experimental period.

Climatic Conditions

To assess the potential impact of climatic factors, particularly temperature and rainfall, on the experiment, monthly climate data were obtained from bulletins and publications of the Institute of Geosciences for the years 2020-2023. Additionally, to provide a broader context, climate data spanning the preceding decade (2013-2023) were analyzed. (Maho et al., 2023)

Analysis of the climate data revealed significant interannual variability in temperature and precipitation patterns. A consistent trend of increasing temperatures throughout the year was observed. However, no clear trend in precipitation amounts was discernible. This observed pattern of rising temperatures aligns with broader climate change trends observed over the past decade (2013-2023).

The period from April to June is particularly critical for plant productivity, as it encompasses key developmental stages such as flowering, pod set, and grain filling. During this period, average temperatures for the study years (2020-2023) were notably higher compared to the 30-year average (1991-2020). Specifically, average temperatures for May and June were 1.6°C and 0.3°C higher, respectively. Overall, the average temperature increase for the April-June period during the experimental years was 1.4°C above the 30-year average.

Monthly rainfall patterns during the April-June period also exhibited considerable variation. The highest cumulative rainfall for this period occurred in 2023, while the lowest was recorded in 2021.

Table 1. Weather conditions at the experiment site.

Month	**2020**	**2021**	**2022**	**2023**	**1991–2020**	**2020**	**2021**	**2022**	**2023**
Total precipitation (mm)						Deviations from the total precipitation of **1991–2020** (%)			
April	54	53	78	66	75	-28	-29.3	4	−12
May	41	18	16	110	67	-38.8	-73.1	−76.1	64.2
June	41	85	80	142	34	20.6	150	135.3	317.6
April– June	136	156	174	318	176	-22.7	-11.4	−1.1	80.7
Average precipitation (mm)			2020-2023	196	176	11.4			
Average air temperature (°C)						Deviations from the average air temperature of **1991–2020** (°C)			
April	9.8	9.4	10	9.2	8.8	1	0.6	1.2	0.4
May	15	15.8	16.7	13.6	13.4	1.6	2.4	3.3	0.2
June	18.3	19.4	20.9	18.8	18	0.3	1.4	2.9	0.8
April– June	14.4	14.9	15.9	13.9	13.4	1	1.5	2.5	0.5
Average			2020-2023	14.8	13.4	1.4			

Field Observations and Measurements

To evaluate the influence of row spacing and planting density on plant development and yield, comprehensive field observations and measurements were conducted throughout the growing season.

Phenological Observations: Key phenological stages were monitored visually and recorded, including:

1. Planting date
2. Date of germination (beginning and complete)
3. Flowering (beginning and ending)
4. Seed maturation (beginning and complete)

The initiation of each phenological stage was defined as the point when 10% of the plants within a plot exhibited the characteristic, while complete stages were recorded when 75% of plants reached the specific stage. Flowering completion was documented when 90% of plants had finished flowering, and full seed maturation was defined as the point when 75% of plants had the majority of pods ripened.

Biometric Measurements: Biometric measurements were taken from designated plants **at full seed maturation to assess plant growth and yield components. These measurements** included:

1. Number of pods with seeds and without seeds per plant
2. Number of grains per plant
3. Height of the first pod from the ground
4. Pod length, width, and thickness
5. Number of grains per pod
6. Grain-to-pod ratio (in the analyzed plants)
7. Pea seed yield was determined at 14% moisture content.

Agronomic Practices: Consistent agronomic practices were applied across all experimental plots to ensure uniformity, with variations only in row spacing and planting density. Key practices included:

- **Tillage:** Soil tillage was performed at a depth of 25-30 cm, followed by milling to create a friable seedbed.

- **Basic Fertilization:** Prior to planting, 50 kg/ha of phosphorus (P) and 70 kg/ha of potassium (K) were applied as base fertilizers.

- **Planting:** Planting was conducted manually between September 20th and 30th at the designated row spacings and planting densities. Seeds were sown at a depth of 3-5 cm.

- **Cultural Practices:**

- **Weed Control:** Two to three weeding operations were performed at 10–12-day intervals, as field conditions allowed.

- **Supplementary Fertilization:** One application of supplementary nitrogen fertilizer (1 kg/ha) was applied during the second weeding. The specific fertilizer rate was determined based on soil nutrient analysis

Data analysis methods

Data were collected from the two-factor experiment in randomized complete blocks with four replications. Two-way analysis of variance (ANOVA) was used for statistical evaluation; while the significance of the results was verified with the HSD (honestly significant difference) Tukey test at the level of significance $\alpha = 0.01$ and 0.05.

RESULTS AND DISCUSSION

The influence of the factors taken in the study (factor number of plants and distance between rows) was evaluated after processing the experimental data collected in 3 years. At the same time, the change in plant productivity as a result of climatic factors was evaluated.

Pea Yield Components

Indicators of pea yield components were significantly influenced by both environmental conditions and agronomic practices, specifically row spacing and planting density. (Table 2).

The average number of pods per plant varied across the study years, ranging from 2.4 in 2021 to 2.79 in 2023. This variation aligns with the observed rainfall patterns during the April-June period, with 2023 receiving more favorable rainfall for pea production. This finding is consistent with previous research highlighting the critical role of rainfall during this key developmental period. (Karadaş and Ceyhan, 2023). Climatic characteristics, particularly precipitation during April-June when vegetative growth is most active, significantly influence pea yield components.

Climatic variations observed in this study led to significant changes in green mass yield, number of seeds per pod, and seed weight per pod. However, no significant differences were observed in the number of pods per plant or the weight of 1000 seeds. Similarly, (Prusinski and Borowska, 2022)found that rainfall distribution during the growing season was the most critical factor influencing pea yield component values.

Row spacing significantly impacted yield component traits. Wider row spacing (30 cm) resulted in a significantly higher number of pods per plant, seeds per pod, seed weight per pod, and 1000-seed weight compared to narrower spacing (20 cm).

Planting density also played a significant role in shaping yield components. The highest average number of pods per plant (3.17) was observed at a planting density of 60 plants per m^2. However, the highest values for the number of seeds per pod, seed weight per pod, and 1000-seed weight were recorded at a higher planting density of 80 plants per m^2.

Table 2. Pea yield components depending on row spacing and plant density across the years.

Factor		No. of Pods per Plant	No. of Seeds per Pod	Seed Weight per Pod (g)	Weight of 1000 Seeds (g)
	2021	2.4	3.35	0.491	146.6
	2022	2.38	3.42	0.513	150
	2023	2.79	3.38	0.518	153.4
Row spacing (R)	20	2.19	3.37	0.495	147
	30	2.73	3.41	0.525	154
	40	2.57	3.37	0.502	149
Planting density (D)	60	3.17	3.34	0.495	148.3
	80	2.59	3.41	0.515	151
	100	2.04	3.40	0.512	150.7
	Mean	2.54	3..383	0.509	150
	Y	ns	**	**	ns
	R	ns	*	*	ns
	D	*	**	**	ns
	Y × R	ns	**	**	ns
	Y × D	*	**	**	ns
	R × D	ns	**	**	ns
	Y × R × D	ns	ns	ns	ns

Values of a parameter followed by the same letter did not differ significantly between the year, row spacing, and planting density (ANOVA followed by Tukey's HDS test, $p < 0.05$) ANOVA results: ** $p < 0.01$; * $p < 0.05$; ns, not significant.

1. No. of Pods per Plant
2. No. of Seeds per Pod
3. Seed Weight per Pod (g)
4. Weight of 1000 Seeds (g)

Pea Seed Yield

In our study, data processing shows that the distance between rows has a significant effect on the yield of pea seeds. The plants show the highest seed productivity at a distance of 30 cm with 1148.7 kg/ha and the lowest at a distance of 20 cm with 869.3 kg/ha. At the width between the rows of 20 cm only resulted in an increase in the biomass yield of peas.

Planting density had a significant effect on plant productivity. The plants show the highest seed productivity at the planting density of 80 seeds/m2 and the lowest at the planting density of 60 seeds/m^2. The same position is taken by researchers Bitew et al, 2014 who emphasize that the space between and within the rows together leads to growth and decreases seed yield. (Inter and intra row spacing together leads to increase and decrease the seed yield)

Important changes in the yield of pea seeds also appear due to climatic factors. The change from year to year in temperature and precipitation showed significant changes in the yield of pea seeds. Significant differences are observed in the yield of pea seeds in terms of the interaction of the factors (R × D), distance between rows (R) and planting density (D).

The collected data were systematized for the purpose of statistical analysis. General data are presented in Table 3.

Table 3. Table of the influence of three factors: climate (Y), Row spacing factor (R), Planting density factor (D) on the yield of pea grains (kg/ha)

Factor Years (Y)	Factor Row spacing (R)	Factor Planting density (D)			Amount	Avarege
		60 seeds	80 seeds	100 seeds		
2021	20 cm	782	854	862	2498	832.6
	30 cm	944	1087	1045	3076	1025.3
	40 cm	823	957	914	2694	898
2022	20 cm	821	865	884	2570	856.7
	30 cm	996	1146	1124	3266	1088.7
	40 cm	860	1058	1032	2950	983.3
2023	20 cm	896	906	954	2756	918.7
	30 cm	1186	1430	1380	3996	1332
	40 cm	1167	1285	1204	3656	1218.7
Amount		8475	9588	9399	27462	9154.3
Avarage		941.7	1065.3	1044.3	3051.3	1017.1

To evaluate the related influence of the distance between the rows (R) and the planting density (D), the three-year experimental data obtained from the processing are presented in Table 4.

Table 4. Table of the influence of two factors on the yield of pea grains (kg/ha) Row spacing factor (R) and Planting density factor (D).

Factor Row spacing (R)	Factor Planting density (D)			Amount	Avarage
	60 seeds	80 seeds	100 seeds		
20 cm	833	875	900	2608	869.3
30 cm	1042	1221	1183	3446	1148.7
40 cm	950	1100	1050	3100	1033.3
Amount	2825	3196	3133	9154	3051.3
Avarage	941.7	1065.3	1044.3	3051.3	1017.1

Two-way analysis of variance (ANOVA) was used for statistical evaluation; while the significance of the results was verified with the HSD (honestly significant difference) Tukey test at the level of significance α = 0.01 and 0.05. The data analyzed with the ANOVA method are presented in Table 5.

Table 5. Effect of row spacing and planting density on the seed yield (kg·ha^{-1}) in 2021–2023

Factor		**Year**			**Mean**
		2021	**2022**	**2023**	
Row spacing (R)	20	832.6	856.7	918.7	869.3
	30	1025.3	1088.7	1332	1148.7
	40	898	983.3	1218.7	1033.3
Planting density (D)	60	849.7	892.3	1083	941.7
	80	966	1023	1207	1065.3
	100	940.3	1013.3	1179.3	1044.3
	Mean	918.6	976.2	1156.5	1017.1
	Y	**	**	**	**
	R	**	**	**	**
	D	**	**	**	**
	R × D	*	*	*	*

Values of a parameter followed by the same small letter in columns and big ones in rows did not differ significantly (ANOVA followed by Tukey's HDS test, $p < 0.01$ and 0.05). ANOVA results: * $p < 0.05$; ns, not significant.

Our study revealed a significant effect of both rows spacing (R) and planting density (D) on pea seed yield. The highest average seed yield (1148.7 kg/ha) was achieved with a 30 cm row spacing. Reducing the row spacing from 40 cm to 20 cm increased pea biomass yield but did not result in a corresponding increase in seed yield. This finding aligns with the observations of (Karadeniz and Bengisu, 2022), who reported similar results.

Factor D, Planting density also significantly influenced pea seed yield. The highest average seed yield (1065.3 kg/ha) was obtained at a planting density of 80 seeds/m^2.

Pea Protein Yield

There is no significant effect on average protein yield of pea by R factor row spacing and D factor planting density. The average protein content in pea grains for the years 2021-2023 was on average 26.8%.

DISCUSSION

Structural indicators of pea yield depended significantly on row spacing and planting density.

The average number of pods per plant was 4.6, and in 2023, the most favorable year in terms of April - June rainfall, it was higher than in the less favorable years 2021 and 2023, similar to pea seed yield. Favorable rainfall conditions affect seed yield, and number of seeds per pod and seed weight per pod.

The R factor, row spacing showed statistically significant influence on the number of seeds per pod, seed weight per pod and seed yield of fodder pea.

Factor D planting density showed statistically significant effect on number of pods per plant, number of seeds per pod, seed weight per pod and seed yield of forage pea.

The R x D interaction showed statistically significant effects on the number of seeds per pod, seed weight per pod and seed yield of forage pea.

According to (Salter and Williams, 2015), seed weight per plant in pea decreased regularly with increasing planting density which is consistent with our experimental data.

Among the many factors affecting pea grain yield, plant density and row spacing are important. (Bitew et al, 2014)Regarding the distance and number of plants with the best influence on the yield of pea grains, different researchers have different conclusions. We think that in this regard these conclusions are not contradictory since the yield of pea grains is significantly affected from cultivar qualities, planting time as well as climatic factors which show variability from area to area.

The optimal density of plants which is determined by the distance between the rows and the number of seeds sown from a biological point of view is explained by intra and inter competition between plants for light and food factors. (Sibhatu et al, 2016)

Therefore, it is very important for the production of legumes to find the optimal density of plants, where the competition between plants does not affect the yield reduction.

According to a wider row spacing of 60 cm resulted in a significantly higher number of pods/plants, compared to a 30 cm row spacing.

A lower plant density increased the number of pods/plants, and a higher plant density decreased the amount of pods/plant. (Ibrahim et al, 2019)

Reducing the distance of planting within the row, determined a higher plant growth, which can be explained by the competition of plants for light. Similar results were reported by (Yucel 2013)

Planting density had a significant effect on most structural components of pea yield. Number of pods, seeds per pod and seed weight per pod were significantly higher when peas were planted in 30 cm rows than in 20 and 40 cm rows.

CONCLUSIONS

Forage pea productivity is affected by several factors. Plant density and row spacing are the most important.

Plant density is determined by the number of seeds planted per m^2. Plant density is one of the factors that affects the morphological and productive development of plants. From a biological point of view, the density of plants affects the competition between plants for lighting and food elements.

The optimal distance between rows also varies between cultivars of the same species. It is conditioned by the different biological characteristics of the cultivars and by other agrotechnical factors. Cultivation of fodder pea "Voskopoja" in hilly lands and in the absence of irrigation is another element that shows influence.

Optimum density in fodder pea planting ensures better competitive ability with weeds by increasing interspecific competition.

The R factor, row spacing showed statistically significant influence on the number of seeds per pod, seed weight per pod and seed yield of fodder pea.

Factor D planting density showed statistically significant effect on number of pods per plant, number of seeds per pod, seed weight per pod and seed yield of forage pea.

The R x D interaction showed statistically significant effects on the number of seeds per pod, seed weight per pod and seed yield of forage pea.

ACKNOWLEDGMENTS

The authors thank: "Fan S. Noli" University, Faculty of Agriculture, Department of Agronomy, Korça, Albania for the support.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Bitew, Y., Asargew, F., and Beshir, O., 2014. Effect of plant spacing on the yield and yield component of Field Pea (Pisum sativum L.) at Adet, North Western Ethiopia. *Agriculture, Forestry and Fisheries*, 3(5), 368-373.

Ditzler, L., Apeldoorn, D., Pellegrini, F., Antichi, D. and Bàrberi, P., 2021. Current research on the ecosystem service potential of legume inclusive cropping systems in Europe. A review *Agronomy for Sustainable Development* 41: 26 pp 1-13.

Ibrahim, D. M., Mohamed, S. J., and Ahmed, S. M., 2019. Effects of plant density on seed yield and it's components of two pea (Pisum sativum L.) cultivars under rainfed condition in Sulaimani province. *Journal of Kerbala for Agricultural Sciences*, 6(1), 36-48.

Kachout, S. S., Ennajah, A., Srarfi, F. and Zoghlam, A., 2021. Salt and drought stress effects on germination and seedling growth on field pea (Pisum sativum L.). *Journal of new sciences, Agriculture and Biotechnology*, Volume 81 (7), 4745-4757.

Karadaş, C. and Ceyhan E., 2023. Determination of the effects of organic and chemical fertilization on grain yield and some agricultural characteristics of pea. *Selcuk Journal of Agriculture and Food Sciences*, 37(2), 419-429.

Karadeniz, E. and Bengisu, G., 2022. Effects of Row Spacing on Yield and Quality of Forage Pea (Pisum sativum ssp. arvense). *Turkish Journal of Range and Forage Science*, 3(1): 30-35 doi: 10.51801/turkjrfs.897275.

Knott, C. and Belcher S., 1998. Optimum sowing dates and plant populations for winter peas (Pisum sativum). *Journal of Agricultural Science, 131*, 449-454.

Krizmanić, G., Tucak, M., Brkić, M., Marković, M., Jovanović, V. and Čupić, T., 2020. The impact of plant density on the seed yield and the spring field pea's yield component. *Poljoprivreda*, Volume 26, 25–31. [Google Scholar] [CrossRef].

Kumar, K. and Goh K. M., 2000. Biological nitrogen fixation, accumulation of soil nitrogen and nitrogen balance for white clover (Trifolium repens L.) and field pea (Pisum sativum L.) grown for seed. *Field Crops Research,* Volume 68, Issue 1, Pages 49-59.

Maho, A., Mero, Gj. and Maho, F., 2023. Changes in Agro-climatic Indicators in the Korça Region. *Current Applied Science and Technology*, Vol. 23 No. 4 Pages: 1–10.

Mirzad , M., Kazemi, H., Sheikh, F., Klug, H. and Gherekhloo, J., 2023. Assessment and quantification of some short term ecosystem services in garden pea field. *Journal of Cleaner Production*. Volume 414, 15 August 2023, 137464.

Prusiński, J. and Borowska, M., 2022. Effect of Planting Density and Row Spacing on the Yielding and Morphological Features of Pea (Pisum sativum L.). *Agronomy*, Volume 12 Issue 3 10.3390/agronomy12030715

Sallaku, G., Liko, J., Rada, Z. and Balliu, A., 2016. The effects of legume crops (pea and faba bean) on soil nutrients availability and yield parameters of subsequent cabbage crops under organic production conditions. *Journal of environmental science & engineering*, 619-625.

Salter, J. and Williams, J.B, 2015. The effect of irrigation on pea crops grown at different plant densities. *Journal of Horticultural Science*, 42, 59–66. [Google Scholar] [CrossRef]

Sibhatu, B., Berhe, H., Gebrekorkos, G., and Abera, K. 2016. Determination of planting spacing for improved yield and yield components of Dekoko (Pisum sativum var. abyssinicum) at Raya Valley, Northern Ethiopia. *African Journal of Plant Science*, 10(8), 157-161. https://doi.org/10.5897/AJPS2016.1428

Trupa, A., Osmane, B. and Konosonoka, I., 2018. Fodder beans and peas in the diet of dairy cows. *Agronomy Research,* 16(4), 1905-1914, https://doi.org/10.15159/AR.18.160.

Volpelli, L. A., Comellini, M., Masoero, F., Moschini, M., Pietro Lo Fiego, D. and Scipioni, R., 2010. Faba beans (Vicia faba) in dairy cow diet: effect on milk production and quality. *Italian Journal of Animal Science,* Volume 9, Issue 1.

Yucel, D. O., 2013. Optimal intra-row spacing for production of local faba bean (Vicia faba L. major) cultivars in the Mediterranean conditions. *Pakistan Journal of Botany*, 45(6): 1933-1938.

OPTIMIZING SOLID-STATE FERMENTATION OF OLIVE POMACE WITH ENZYMES FOR IMPROVED NUTRIENT CONTENT

Emrah GÜNGÖR,[1] Şevket ÖZLÜ[1], Bahar GÜNGÖR[2], Aydın ALTOP[1], Güray ERENER[1]

[1] Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Turkiye
[2] Ondokuz Mayıs University, Faculty of Science, Department of Molecular Biology and Genetics, Samsun, Turkiye

Corresponding author e-mail: emrah.gungor@omu.edu.tr

ABSTRACT

This study investigated the effects of exogenous enzymes on the nutrient composition of fermented olive pomace in the solid-state fermentation method. The research was conducted in a 3 x 2 factorial experiment, with three different fermentation periods (3, 5 and 7 days), two different enzyme additions (yes, no) in each period, and five replicates in each group for a total of 35 samples, including the control group. Before fermentation, the olive pomace was ground to a particle size of 1 mm and added to the fermenter. Subsequently, 100 g of the ground olive pomace and 500 ml of distilled water were added to each fermenter. The enzyme used in this study comprises a combination of phytase, xylanase, and beta-glucanase. The enzyme was then added to a quantity of 1 g to each fermentation media and sterilized via UV light. 1 ml of the *L. acidophilus* culture (10^8 cfu/ml) was added to the sterilized fermenters. The inoculated samples were fermented at 30 °C for the specified duration (3–5–7 days). The study concluded that the nutrient composition of olive pomace was positively affected. Overall, crude protein and ether extract levels increased significantly ($P<0.001$). Additionally, a fermentation time x enzyme interaction was observed regarding nutrient composition ($P<0.001$). At the end of fermentation, the highest crude protein level was determined in fermented samples for three days with enzyme supplementation or five days without enzyme supplementation ($P<0.001$). The lowest crude fiber level was observed in the non-fermented sample ($P<0.001$). In contrast, the second lowest crude fiber level in the fermented samples was observed in the sample fermented for seven days without enzyme supplementation ($P<0.001$). The most effective fermentation time of olive pomace with *L. acidophilus* was three days with enzymes.

Keywords: Solid-state fermentation, olive pomace, *L. acidophilus*, enzyme

INTRODUCTION

Meat and meat products are essential protein sources for ensuring adequate and balanced nutrition for the human population. Due to global population growth, demand for meat products is projected to increase by approximately 58% (Makkar et al., 2014). Turkey's broiler chicken production is critical in meeting the rising global demand. However, the production rate must be increased to meet the increasing demand for broiler chickens. This increase will also result in an increased demand for feedstuffs. Furthermore, given that some feedstuffs used in animal nutrition are also used in human nutrition, it is vital to develop alternative sources.

In poultry production, feed costs represent approximately 60-70% of total costs. Using alternative materials and feed additives instead of the feedstuffs typically used in animal feeds can help reduce feed costs. Antibiotics, used as growth factors in broiler feeds, can increase the risks and leave residues in the product. Consequently, since 2006, various alternative additives have been developed to enhance performance in broiler chickens, protecting intestinal health and providing additives. Among these additives, the use of various raw materials classified as agricultural waste has been a topic of interest in recent years.

The olive (*Olea europaea*) is a plant species belonging to the olive family (*Oleaceae*). This tree, native to the Mediterranean climate, exhibits a shrub form or a structure that can reach a height of up to 10 meters. The plant exhibits a densely branched, spreading top and evergreen leaves. The global olive tree population is estimated to be approximately 900 million, distributed across 11 million hectares. This yields an average of 22 million tonnes of olives annually (FAO, 2023). The countries that produce the majority of olives are Spain, Italy, Greece, Turkey, Tunisia and Morocco.

Fermentation effectively increases nutrient availability in various feed materials, by-products, waste products, and medicinal plants (Altop et al., 2017; Altop et al., 2018; Güngör et al., 2021). This process involves the chemical change caused by microorganisms on organic matter, which results in an improved nutrient composition. Several studies have demonstrated that agricultural waste can be utilized in animal nutrition through fermentation, enhancing animal performance, and various other parameters (Wang et al., 2018; Saleh et al., 2021). Furthermore, fermentation has been demonstrated to enhance the properties and bioavailability of phytochemicals, which are responsible for the biological properties of plants. These include antioxidant, antimicrobial, and anti-inflammatory activity (Nawaz et al., 2011; Yang et al., 2012). This study investigated the effects of exogenous enzymes on the nutrient composition of fermented olive pomace in the solid-state fermentation method.

MATERIAL AND METHOD

The research was conducted in a 3 x 2 factorial experiment, with three different fermentation periods (3, 5 and 7 days), two different enzyme additions (yes, no) in each period, and five replicates in each group for a total of 35 samples, including the control group. The experimental plan of the research is presented in Table 1.

Table 1. Plan of the research

		Days			
		0 days	3 Days	5 days	7 days
Enzyme	With enzyme		Fermented with enzyme for 3 days	Fermented with enzyme for 5 days	Fermented with enzyme for 7 days
	Without enzyme	Control	Fermented without enzyme for 3 days	Fermented without enzyme for 5 days	Fermented without enzyme for 7 days

The olive pomace used in the study was obtained from a local business, and the samples were dried at 75 °C. The samples were stored at 4 °C until use in the study. The microbial inoculant, *L. acidophilus* (ATCC 4356), was obtained from the American Type Culture Collection (ATCC).

Before fermentation, the olive pomace was ground to a particle size of 1 mm and added to the fermenter. Subsequently, 100 g of the ground olive pomace and 500 ml of distilled water were added to each fermenter. To facilitate microbial growth, 85.5 g of nutritional salt (glucose:urea:(NH4)2SO4:peptone:KH2PO4:MgSO4.7H2O = 4:2:6:1:4:1) was added to 1 lt of distilled water (Cao et al., 2012). Subsequently, the samples were sterilized at 121 °C for 15 minutes. The enzyme used in this study was obtained from a private company and comprises a combination of phytase, xylanase, and beta-glucanase. The enzyme was then added to a quantity of 1 g to each fermentation media and sterilized via UV light.

L. acidophilus was incubated in a shaking incubator at 30 °C and 120 rpm for 48 hours using MRS broth (Güngör & Erener, 2020). Then, 1 ml of the L. acidophilus culture (10^8 cfu/ml) was added to the sterilized fermenters. The inoculated samples were fermented at 30°C for the specified duration (3–5–7 days). Once the fermentation process had been completed, dry matter, ash, crude protein, crude fiber, and ether extract analyses were conducted by Akyıldız (1984).

The data obtained from the research was subjected to analysis of variance using the GLM ANOVA/MANOVA procedure in the statistical software Statistica (1984) in a 3 x 2 factorial arrangement. This involved three fermentation periods (3, 5 and 7 days), two enzyme additions (yes or no), and a randomized-block design. Once the statistical significance of the differences between the groups had been established ($P < 0.05$), Tukey's HSD test was used to identify the specific group to which the difference applied ($P = 0.05$).

RESULTS AND DISCUSSION

The study concluded that the nutrient composition of olive pomace was positively affected. The results obtained were comparable to those reported by Altop et al. (2019). Overall, crude protein and ether extract levels increased significantly ($P<0.001$). Additionally, a fermentation time x enzyme interaction was observed regarding nutrient composition ($P<0.001$). The crude fiber level, which is an anti-nutritional factor, especially for monogastric animals, decreased ($P<0.001$), while the crude protein and ether extract levels increased ($P<0.001$). At the end of fermentation, the highest crude protein level was determined in fermented samples for three days with enzyme supplementation or five days without enzyme supplementation ($P<0.001$). The nutrient composition of feedstuffs is positively affected by the solid-state fermentation method (Wang et al., 2017; Altop et al., 2018). In a study by Li et al. (2020), soybean meal fermented with *L. acidophilus* demonstrated an increase in crude protein level, consistent with the results mentioned above. The reason for this increase is microbial protein production.

The lowest crude fiber level was observed in the non-fermented sample ($P<0.001$). In contrast, the second lowest crude fiber level in the fermented samples was observed in the sample fermented for seven days without enzyme supplementation ($P<0.001$). The increase in crude fiber in the fermented samples is attributed to the alteration in the nutrient composition of the olive pomace. In a study conducted by Güngör et al. (2017), it was reported that cherry kernels fermented with different subspecies of microorganisms affected the crude fiber levels in different ways. There may have been a relative increase in crude fiber rates due to a lack of microorganism-substrate compatibility or a decrease in other nutrients (carbohydrates).

CONCLUSION

Fermentation has been demonstrated to positively affect the nutrient composition of various agricultural wastes, including olive pomace. This process allows for the increased use of feedstuffs in diets while also conferring additional benefits due to the probiotic effects of microorganisms. This research also supports this result, as crude protein levels increased while ether extract levels were maintained and crude fiber levels decreased. The most effective fermentation time of olive pomace with *L. acidophilus* was three days with enzymes.

REFERENCES

Akyıldız, A. (1984). *Yemler bilgisi laboratuvar kılavuzu.* Ankara: Ankara Üniversitesi Ziraat Fakültesi Yayınları.

Altop, A., Coşkun, İ., Filik, G., Küçükgül, A., Bekiroğlu Genç, Y. ve Erener, G. (2017). Zeytin Yaprağının (*Olea europaea* L.) Besin Madde Kompozisyonu ve Fitokimyasal Bileşenleri Üzerine Farklı Mikrobiyal İnokulantlar ile Fermentasyonun Etkileri. 114R110, *Tübitak Projesi Kesin Sonuç Raporu*.

Altop A, Güngör E, Erener G. *Aspergillus niger* may improve nutritional quality of grape seed and its usability in animal nutrition through solid-state fermentation. International Advanced Researches and Engineering Journal. 2018;2(3):273-277.

Altop, A., Güngör, E., and Erener, G., 2019. Improvement of nutritional quality of some oilseed meals through solid-state fermentation using Aspergillus niger, Turkish Journal of Agriculture-Food Science and Technology, 7, 1411-1414.

Cao, F., Zhang, X., Yu, W., Zhao, L. ve Wang, T. (2012). Effect of feeding fermented Ginkgo biloba leaves on growth performance, meat quality, and lipid metabolism in broilers. Poultry Science, 91 (5). 1210-1221.

FAOSTAT (2023). Birleşmiş Milletler Gıda ve Tarım Örgütü. http://www.fao.org/faostat (Erişim tarihi:05.07.2024).

Güngör E, Erener G. Katı faz fermantasyonunun ceviz küspesinin besinsel kompozisyonu ve in vitro sindirilebilirliği üzerine etkisi. Balevi T, editör. Kanatlıların Beslenmelerinde Son Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2023. p.64-9.

Güngör, E., Altop, A., Öztürk, E., and Erener, G., 2017. Nutritional changes of sour cherry (Prunus cerasus) kernel subjected to Aspergillus niger solid-state fermentation, Tekirdağ Ziraat Fakültesi Dergisi.

Gungor, E. ve Erener, G. (2020). Effect of dietary raw and fermented sour cherry kernel (*Prunus cerasus* L.) on growth performance, carcass traits, and meat quality in broiler chickens. *Poultry Science*, 99 (1). 301-309.

Li Y, Guo B, Wu Z, Wang W, Li C, Liu G, Cai H. Effects of fermented soybean meal supplementation on the growth performance and cecal microbiota community of broiler chickens. Animals. 2020;10(6):1098.

Makkar, H. P., Tran, G., Heuzé, V. ve Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, 197, 1-33.

Masuda, T., Hidaka, A., Kondo, N., Ura, T., & Itoh, T. (2005). Intracellular enzyme activities and autolytic properties of Lactobacillus acidophilus and Lactobacillus gasseri. *Food science and technology research*, *11*(3), 328-331.

Özlü Ş, Altop A. Çeşitli probiyotik mikroorganizmalar tarafından fermente edilmiş zeytin yapraklarının besin değeri ve in vitro sindirilebilirliği. Balevi T, editör. Kanatlıların

Beslenmelerinde Son Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2023. p.70-4.

Saleh, A. A., Shukry, M., Farrag, F., Soliman, M. M. ve Abdel-Moneim, A. M. E. (2021). Effect of feeding wet feed or wet feed fermented by *Bacillus licheniformis* on growth performance, histopathology and growth and lipid metabolism marker genes in broiler chickens. *Animals*, 11 (1). 83.

Wang JH, Guo H, Zhang TR, Wang H, Liu BN, Xiao S. Growth performance and digestion improvement of juvenile sea cucumber Apostichopus japonicus fed by solid-state fermentation diet. Aquaculture Nutrition. 2017;23(6):1312-1318.

USE OF OLIVE (Olea europaea L.) AND MULBERRY LEAVES (Morus alba L.) IN BROILER DIETS

Güray ERENER[1], Şevket ÖZLÜ[1], Emrah GÜNGÖR[1], Bahar GÜNGÖR[2], Aydın ALTOP[1]

[1] Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Turkiye
[2] Ondokuz Mayıs University, Faculty of Science, Department of Molecular Biology and Genetics, Samsun, Turkiye

Corresponding author e-mail: bahargaripoglugungor@gmail.com

ABSTRACT

Antibiotic growth promoters have long been used in animal nutrition. However, this widespread and intensive use has led to the emergence of antibiotic-resistant bacterial species that threaten human health and life. For this reason, antibiotic growth promoters in poultry feeding have been banned in many countries, including the European Union, since 2006. The ban on antibiotics has led to aggravation of bacterial infections and decreased performance in poultry. For this reason, research has focused on finding natural antibiotic alternatives. For this purpose, various growth-promoting alternatives such as beneficial microorganisms (probiotics and prebiotics) and herbal additives and their extracts have been tested and used in the nutrition of poultry and livestock. Herbal additives, also known as phytobiotics, are phytochemical compounds and are substances obtained from the leaves, roots, seeds, flowers, buds, bark of plants or their extracts and have pharmacological effects. Leaves of olive and mulberry trees are among the plants used as phytobiotics in poultry feeding. In this study, we tried to give information about the studies in the last fifteen years on the use of tree-shaped olive and mulberry leaves, which are used as tea, in the nutrition of broiler diets.

Keywords: Olive leaf, mulberry leaf, phytobiotics, broiler

INTRODUCTION

Intensive use of antibiotics for animal nutrition has led to the emergence of antibiotic-resistant bacterial species. For this reason, the use of antibiotics for growth purposes in poultry feeding has been banned in many countries, including the European Union, since 2006 (Alagawany et al. 2020). After the ban, various growth-promoting alternatives such as beneficial microorganisms (probiotics and prebiotics), many plants, herbal additives and their extracts were tested as safe alternatives to antibiotics, and they began to be used as natural feed additives in the nutrition of poultry and farm animals (Abd El-Hack et al. 2017, 2022; Chen et al., 2019, Rahman et al., 2022). Phytogenic feed additives, among these feed additives, have a great potential in the poultry industry.

Phytogenic feed additives are natural plant-based feed additives used in animal nutrition and are obtained from herbs, spices, other plants and their extracts and essential oils. In other words, herbal supplements, also known as phytobiotics, are phytochemical compounds obtained from the leaves, roots, seeds, flowers, buds, bark or their extracts of plants and have been used in human medicine since ancient times and show pharmacological effects (Martel et al., 2020, Deminicis et al., 2021, Rafeeq et al., 2023). The chemical compositions of phytogenic feed additives vary significantly depending on the effects of climatic conditions, location where plants are grown, harvest stage, as well as storage conditions of different plant extracts. Phytogenic feed additives can also be defined as plant-derived compounds that are added to animal feed to increase animal productivity by improving host digestibility, increasing nutrient absorption, and ensuring the elimination of various pathogens from the animal intestine (Samantaray and Nayak, 2021).

OLIVE (*Olea europaea* L.) LEAF

Olea europaea L., which is an evergreen tree member of the Oleaceae family and covers a total area of 10.8 million hectares, is grown in 41 countries, especially in the Mediterranean basin. The leaves (An agricultural waste) are gathered throughout the year. Olive leaf has all same healthful qualities of olive oil without the fat and in higher concentrations. It has antioxidant properties that protect the body from the continuous activity of free radicals. Olive leaves, especially leaf extract, are used as additives in medicine, cosmetics and many food products. Olive tree is one of the plants that has attracted attention recently (Cayan and Erener, 2015). Olive leaf has many biological activities, including phenolics and flavonoids, as a low-cost essential source of antimicrobials. Over the past few decades, numerous studies involving green technologies have been conducted to isolate active compounds from olive leaves (Selim et al., 2022). Olive leaves are agricultural residues obtained from olive trees (*Olea europea* L.) during fruit harvest. Olive leaves are rich in phenolic compounds such as tyrosol and hydroxytyrosol, especially oleuropein (Silva et al., 2006, Jabri et al., 2017). Additionally, it has immunostimulating, antibacterial (Bisignano et al., 2001), antifungal, anti-viral (Fredrickson, 2000), anti-inflammatory, antibacterial activity (Korukluoğlu et al., 2010) and antioxidant properties (Mujić et al., 2011; Hamad , 2015) has various pharmacological properties.Addition of olive leaves to broiler mixes resulted in increased live weight and improved feed utilization (Fayed et al., 2009; Erener et al., 2020 and Lee-Huang et al., 2011).

El-Damarawy et al (2013) observed that the addition of 2.0% olive leaves to Mandarah chick rations improved performance and most of the immunological and biochemical properties.

MULBERRY (*Morus alba* L.) LEAF

The increasing costs of conventional feedstuffs like corn, soybean meal and fish meal for poultry diets is pushing to find less expensive alternatives. One possible feed alternative is tree fodder mulberry leaves (*Morus alba*). Mulberry belongs to the genus *Morus* contains 16 species family of Moraceae and 11 species are found in China. Genus Morus is one of such example that consists of over 150 species, among these *Morus alba* L. is dominant (Ustundag and Ozdogan, 2015). The mulberry plant (*Morus alba*) is a fast-growing shrub, deciduous perennial woody plant, and its leaves are utilized as the primary food source for silkworms. Mulberry grows well in the tropics and subtropics, and is reported to have excellent nutritional value. Mulberry leaves are very rich in protein (15-35%), minerals [2.42-4.71% calcium (Ca); 0.23-0.97% phosphorus (P)] and metabolizable energy (1130-2240 kcal/kg) with absence of or negligible anti-utritional factors. The amino acids composition of mulberry leaf meal indicates it is a good source of essential amino acids especially lysine 1.80% and leucine 2.58% (Al-Kirshi et al., 2009, Al-Kirshi et al 2013). Mulberry leaves and their extracts have been used in folk medicine due to their therapeutic properties, particularly for their anti-inflammatory, anti-diabetic, and antioxidant properties (Chen et al., 2019). The active components of mulberry leaves can also help regulate the antioxidant capacity of laying hens. Although mulberry leaves are widely used in poultry and livestock, their effects on growth, production performance, gut microbiota, and immunological parameters remain unclear (Geng et al.,2024). Its leaves are considered as a high-quality forage plant resource because of its phytosterols, flavonoids, alkaloids, polysaccharides and other bioactive substances. However, due to the high content of crude fiber in mulberry leaves and branches and the presence of anti-nutritional factors such as tannin, the excessive addition of mulberry leaves and branches may be affect the production performance and health of livestock and poultry (Ding et al., 2021).

CONCLUSION

The ban on the use of antibiotics in many countries due to their side effects on both poultry and humans has made the use of herbal natural growth promoters that do not have any negative effects important. In recent years, many studies have been conducted to evaluate the effect of various plants as alternatives to antibiotics on animal production performance and quality of products. Various parts, extracts and essential oils of plants are added to poultry feed to increase appetite, stimulate the secretion of endocrine and digestive enzymes, and stimulate antimicrobial, anti-inflammatory, antioxidative and immune activity. In this study, we tried to give information about the use of tree-shaped olives (*Olea europaea*) and mulberry leaves (*Morus* spp.), which are used as tea in the mixed feeds of broiler chickens, in the nutrition of broiler chickens. Different results have been obtained in studies on the use of olive and mulberry tree leaves. The difference between the results may be due to the doses of plant leaves used in the experiments, the geographical location where the plants are grown, the type of plant, the characteristics of the soil in which it is grown, weather conditions, altitude, season, harvest procedure, storage conditions, phenolic substance contents of the plants, physiological states of the animals, growing conditions and experimental approaches. Therefore, before deciding to use olive and mulberry leaves in the nutrition of broiler chickens, it is necessary to take into consideration the above-mentioned issues in order to get high efficiency from broiler chickens.

REFERENCES

Abd El-Hack, M.E.,. El-Shall, N.A., El-Kasrawy, N.I., El-Saadony, M.T., Shafi, M.E, Zabermawi, N.M., & Alshilawi, M.S., et al. 2022. The use of black pepper (Piper guineense) as an ecofriendly antimicrobial agent to fight foodborne microorganisms. Environmental Science and Pollution Research International, 29 (8): 10894–10907

Abd El-Hack, M.E., Mahgoub, S.A., Alagawany, M., & Ashour, E.A. 2017. Improving productive performance and mitigating harmful emissions from laying hen excreta via feeding on graded levels of corn ddgs with or without bacillus subtilis probiotic. Journal of Animal Physiology and Animal Nutrition 101 (5): 904–913.

Alagawany, M.M., El-Hindawy, M., Mohamed, L.A., Bilal, R.M., & Soomro, J., 2020. The use of cold pressed oils as eco-friendly alternatives for antibiotics in high and low-cp diets of laying japanese quail. Animal Biotechnology 33 (5): 816-823.

Al-Kirshi, R.A., Alimon, A.R., Zulkifli, I., Zahari, M. W., & Sazili, A.Q. 2009. The chemical composition and nutritive value of mulberry leaf as a protein source in poultry diets. In *Proceedings of 1st International Seminar on Animal Industry* 81-85.

Al-Kirshi, R. A., Alimon, A., Zulkifli, I., Atefeh, S., Zahari, M. W., & Ivan, M. 2013. Nutrient digestibility of mulberry leaves (Morus alba). Italian Journal of Animal Science, 12 (2), e36.

Bisignano, G., Lagana, M.G, Trombetta, D., Arena, S., Nostro, A, Uccella, N., Mazzanti, G. & Saija, A. 2001. In vitro antibacterial activity of some aliphatic aldehydes from Oleaeuropaea L. FEMS Microbiology Letters, 198: 9-13.

Cayan, H., & Erener, G., 2015. Effect of olive leaf (Oleaeuropaea) powder on laying hens performance, egg quality and egg yolk cholesterol levels. Asian-Australasian Journal of Animal Science,28:538–54.

Chen, Y., Ni, J., & Li, H., 2019. Effect of green tea and mulberry leaf powders on the gut microbiota of chicken. BMC Veterinary Research, 15(77): 1-6.

Deminicis, R.G.S., Meneghetti, C., Oliveira, E.B., Júnior, A.A.P.G., Filho, R.V.F., & Deminicis, B.B., 2021 Systematic review of the use of phytobiotics in broiler nutrition. Revista de Ciências Agroveterinárias 20 (1): 98-106.

Ding, Y., Jiang, X., Yao, X., Zhang, H., Song, Z., He, X., & Cao, R. 2021. Effects of feeding fermented mulberry leaf powder on growth performance, slaughter performance, and meat quality in chicken broilers. Animals, 11(11): 3294.

El-Damrawy, S.Z.; Khalifah, M.M. & Fares, W.A 2013. Dietary olive leaf and antioxidative status in chickens performance, some physiological traits and immunological responses of Mandarah chicks supplemented olive leaves powder in their diets. Egyptian Poultry Science, 33 (1): 279-287.

Erener, G., Ocak, N., Ozturk, E., Cankaya, S., Ozkanca, R., & Altop, A., 2020. Evaluation of olive leaf extract as a growth promoter on the performance, blood biochemical parameters, and caecal microflora of broilers chickens. Revista Brasileira de Zootecnia,49:e20180300.

Fayed, A. M., El Ashry, M. A., & Hend, A. A. 2009. Effect of feeding olive tree pruning by - products on sheep performance in Sinai. World Journal of Agriculture Sciences, 5(4):436-445.

Fredrickson, W.R.F. &Group, S. Inc. 2000. Method and Composition for Antiviral Therapy with Olive Leaves.United States Patent, 6: 117- 884.

Geng, B., Gao, J., Cheng, H., Guo, G., & Wang, Z. 2024. Effects of dietary mulberry leaves on growth, production performance, gut microbiota, and immunological parameters in poultry and livestock: A systematic review and meta-analysis. Animal Bioscience, 37(6): 1065-1076.

Hamad, I. 2015. Antioxidant Activity and Potential Hepato - Protective Effect of Saudi Olive Leaf Extract. 2nd Int'l Conference on Advances in Environment, Agriculture & Medical Sciences, 72-75.

Jabri, J.,Kacem, H., Yaıch, H., Abıd, K., Kamoun, M., Rekhıs, J., & Malek, A., 2007. Effect of Olive leaves extract supplementation in drinking water on zootechnical performances and cecal microbiota balance of broiler chickens. Journal of new sciences, Sustainable Livestock Management, 4(2): 69- 75

Korukluoglu, M., Sahan, Y.,Yigit, A., Ozer, E.T. & Gücer, S., 2010 Antibacterial activity and chemical constitutions of oleaeuropaea leaf extracts. Journal of Food Processing and Preservation, 34 (3): 383–396.

Lee-Huang, S., Zhang, L., Huang, P.L., Chang, Y.T. & Huang, P.L., 2011. Anti-HIV activity of olive leaf extract (OLE) and modulation of host cell gene expression by HIV-1 infection and OLE treatment. Biochemical and Biophysical Research Communications, 307 (4): 1029-1037.

Martel, J.D., Ojcius, M., Ko,Y.F., & Young, J.D., 2020. Phytochemicals as Prebiotics and Biological Stress Inducers. Trends in Biochemical Sciences 45(6): 462–471.

Mujić, I.; Živković, J.; Nikolić, G.; Vidović, S.; Trutić, N.; Kosić, U.; Jokić, S. & Ruznic, A. 2011. Phenolic compounds in olive leaf extract as a source of useful antioxidants. Croatian Journal of Food Technology, Biotechnologyand Nutrition, 6 (3-4):129-133.

Rahman, M.R.T., Fliss, I, & Biron, E. 2022. Insights in the Development and Uses of Alternatives to Antibiotic Growth Promoters in Poultry and Swine Production. Antibiotics. 11(6):766.

Rafeeq, M., Bilal, R.M., Batool, F., Yameen, K., Farag, M.R., Madkour, M., Elnesr, S.S., El-Shall, N.A., Dhama, K., Nassar, H., Abdel-Raheem, H., & Sotohy, S. 2019. Effect of dietary olive leaves and chamomile flowers powder on the growth performance and carcass traits and of broilers (Sasso Breed). Assiut Veterinary Medical Journal. 65 (163): 58-67

Samantaray, L. & Nayak, Y., 2021. The effectiveness of phytobiotic additives in poultry nutrition Review Article. Strad Research, 8(6):424-428,

Selim, S., Albqmi, M., Al-Sanea, M.M., Alnusaire, T.S., Almuhayawi, M.S., AbdElgawad, H., Al Jaouni, S.K., Elkelish, A., Hussein, S., Warrad, M., & El-Saadony, M.T., 2022. Valorizing the usage of olive leaves, bioactive compounds, biological activities, and food applications: A comprehensive review. Frontiers in Nutrition, 9:1008349.

Silva, S., Gomes, L., Leitao, F., Coelho, A.V., & Vilas Boas, L., 2006. Phenolic compounds and antioxidant activity of Oleaeuropaea L. fruits and leaves. Food Science and Technology International, 12: 385-395.

Ustundag, A. O., & Ozdogan, M., 2015. Usage possibilities of mulberry leaves in poultry nutrition. Scientific Papers. Series D. Animal Science, 58.

IMPROVING NUTRIENT COMPOSITION: ENZYME-ASSISTED BLACK CARROT PULP FERMENTATION

Şevket ÖZLÜ[1], Emrah GÜNGÖR[1], Bahar GÜNGÖR[2], Aydın ALTOP[1], Güray ERENER[1]

[1] Ondokuz Mayıs University, Faculty of Agriculture, Department of Animal Science, Samsun, Turkey
[2] Ondokuz Mayıs University, Faculty of Science, Department of Molecular Biology and Genetics, Samsun, Turkey

Corresponding author e-mail: sevket.ozlu@omu.edu.tr

ABSTRACT

This study investigated the effects of exogenous enzymes on the nutrient composition of fermented black carrot pulp in the solid-state fermentation method. The research was conducted in a 3 x 2 x 5 factorial experiment. Three different fermentation periods were employed: 3-5 and 7 days. Two different enzyme additions (yes, no) were made in each period. Five replicates were conducted for each treatment group, with the total number of samples reaching 35, including the control group. Before fermentation, the black carrot pulp was ground to a particle size of 1 mm and added to the fermenter. To facilitate microbial growth, 85.5 g of nutritional salt (glucose:urea:(NH4)2SO4:peptone:KH2PO4:MgSO4.7H2O = 4:2:6:1:4:1) was added to 1 lt of distilled water. The samples were sterilized at 121 °C for 15 minutes. The enzyme used in this study was obtained from a private company and comprises a combination of phytase, xylanase, and beta-glucanase. The enzyme was then added to a quantity of 1 g to each fermentation media and sterilized via UV light. *L. acidophilus* was incubated in a shaking incubator at 30 °C and 120 rpm for 48 hours using MRS broth. Then, 1 ml of the *L. acidophilus* culture (10^8 cfu/ml) was added to the sterilized fermenters. At the end of the study, the nutrient composition of black carrot pulp exhibited a positive effect. Furthermore, a significant interaction between the factor of fermentation time and the enzyme was observed about the nutrient composition ($P<0.001$). The highest crude protein content was observed in the group that fermented for three days without adding an enzyme ($P<0.001$). The highest level of ash was observed in all samples that fermented for three and five days ($P<0.001$). The lowest level of crude fiber was observed in the non-fermented sample ($P<0.001$). The lowest level was observed in the fermented samples, specifically in the groups that seven days of fermentation without enzymes and three days without enzymes ($P<0.001$). The highest level of ether extract was observed in the non-fermented and Five-day fermented with enzyme addition samples ($P<0.001$).

Key Words: Solid-state fermentation, black carrot pulp, *L. acidophilus*, enzyme

INTRODUCTION

As indicated by Çenesiz et al. (2017), feed costs represent approximately 70% of the overall expenses associated with broiler breeding. In this context, the costs associated with feed significantly impact the rising prices of white meat and eggs. In light of these considerations, it is necessary to investigate the potential of utilizing alternative feedstuffs in broiler feeding. Agricultural waste has significant potential for animal nutrition, with many studies demonstrating its efficacy (Assis et al., 2004; Nazok et al., 2010).

The black carrot (Daucus carota ssp. sativus var. atrorubens Alef.) is a plant cultivated primarily for human consumption. Turkey is a significant producer of this crop (Kamiloglu et al., 2018). The black carrot, cultivated in our country to produce turnips, has been demonstrated to possess antioxidant and anti-inflammatory properties (Gajewski et al., 2007; Ahmad et al., 2019; Yoo et al., 2020). Additionally, it has been demonstrated that the compounds responsible for the carrot's pigmentation function as antioxidants (Chorong et al., 2007). The residue remaining after the squeezing process of black carrot is referred to as pulp and is regarded as agricultural waste in our country, contributing to environmental pollution. An examination of the nutrient content of black carrot pulp reveals the presence of 8-10% crude protein, 20-30% crude fiber, 0-2% ether extract, and 2-5% ash.

Furthermore, its structure's anthocyanins and phenolic compounds render it a highly promising feedstuff for use in animal nutrition. The high level of crude cellulose has a limiting effect, particularly in poultry nutrition. Accordingly, the quality of the feedstuff can be enhanced by implementing the solid-state fermentation method.

Solid-state fermentation (SSF) refers to microorganisms' growth and metabolic activity on moistened solid substrates devoid of free water (Mitchell et al., 2000). SSF enables the conversion of nutrients through the enzymatic interactions of microorganisms on waste material (Steudler et al., 2019). This process has been demonstrated to enhance the nutrient composition of plant material, reduce anti-nutritional compounds, and increase bioavailability. Solid-state fermentation has been demonstrated to improve the digestibility of waste material (Özlü & Altop, 2023) and increase the bioavailability of nutrients (Güngör & Erener, 2023). In studies of fermentation, bacteria, fungi, and yeasts, or combinations thereof, are typically used as inoculants (Adeyemi et al., 2008; Akinfemi, 2010; Ari & Ayanwale, 2012; Ari et al., 2012). This study investigated the effects of exogenous enzymes on the nutrient composition of fermented black carrot pulp in the solid-state fermentation method.

MATERIAL AND METHOD

The research was conducted in a 3 x 2 x 5 factorial experiment. Three different fermentation periods were employed: 3-5 and 7 days. Two different enzyme additions (yes, no) were made in each period. Five replicates were conducted for each treatment group, with the total number of samples reaching 35, including the control group. The experimental plan of the research is presented in Table 1.

The black carrot pulp used in the study was obtained from a local business, and the samples were dried at 75 °C. The samples were stored at 4 °C until use in the study. The microbial inoculant, *L. acidophilus* (ATCC 4356), was obtained from the American Type Culture Collection (ATCC).

Before fermentation, the black carrot pulp was ground to a particle size of 1 mm and added to the fermenter. Subsequently, 100 g of the ground black carrot pulp and 500 ml of distilled water were added to each fermenter. To facilitate microbial growth, 85.5 g of nutritional salt (glucose:urea:(NH4)2SO4:peptone:KH2PO4:MgSO4.7H2O = 4:2:6:1:4:1) was added to 1 lt of distilled water (Cao et al., 2012). Subsequently, the samples were sterilized at 121 °C for 15 minutes. The enzyme used in this study was obtained from a private company and comprises a combination of phytase, xylanase, and beta-glucanase. The enzyme was then added to a quantity of 1 g to each fermentation media and sterilized via UV light.

Table 1. Plan of the research

CONTROL	Enzyme -/1	Enzyme -/2	Enzyme -/3	Enzyme -/4	Enzyme -/5
3 Days	Enzyme +/3-1	Enzyme +/3-2	Enzyme +/3-3	Enzyme +/3-4	Enzyme +/3-5
	Enzyme -/3-1	Enzyme -/3-2	Enzyme -/3-3	Enzyme -/3-4	Enzyme -/3-5
5 Days	Enzyme +/5-1	Enzyme +/5-2	Enzyme +/5-3	Enzyme +/5-4	Enzyme +/5-5
	Enzyme -/5-1	Enzyme -/5-2	Enzyme -/5-3	Enzyme -/5-4	Enzyme -/5-5
7 Days	Enzyme +/7-1	Enzyme +/7-2	Enzyme +/7-3	Enzyme +/7-4	Enzyme +/7-5
	Enzyme -/7-1	Enzyme -/7-2	Enzyme -/7-3	Enzyme -/7-4	Enzyme -/7-5

L. acidophilus was incubated in a shaking incubator at 30 °C and 120 rpm for 48 hours using MRS broth (Güngör & Erener, 2020). Then, 1 ml of the *L. acidophilus* culture (10^8 cfu/ml) was added to the sterilized fermenters. The inoculated samples were fermented at 30°C for the specified duration (3–5–7 days). Once the fermentation process had been completed, dry matter, ash, crude protein, crude fiber, and ether extract analyses were conducted by Akyıldız (1984).

The data obtained from the research was subjected to analysis of variance using the GLM ANOVA/MANOVA procedure in the statistical software Statistica (1984) in a 3 x 2 factorial arrangement. This involved three fermentation periods (3-5-7 days), two enzyme additions (yes or no), and a randomized-block design. Once the statistical significance of the differences between the groups had been established ($P < 0.05$), Tukey's HSD test was used to identify the specific group to which the difference applied ($P = 0.05$).

RESULTS AND DISCUSSION

At the end of the study, the nutrient composition of black carrot pulp exhibited a positive effect. Furthermore, a significant interaction between the factor of fermentation time and the enzyme was observed about the nutrient composition ($P < 0.001$). The highest crude protein content was observed in the group that fermented for three days without adding an enzyme ($P < 0.001$). The highest level of ash was observed in all samples that fermented for three and five days ($P < 0.001$). The lowest level of crude fiber was observed in the non-fermented sample ($P < 0.001$). The lowest level was observed in the fermented samples, specifically in the groups that seven days of fermentation without enzymes and three days without enzymes ($P < 0.001$). The highest level of ether extract was observed in the non-fermented and five-day fermented with enzyme addition samples ($P < 0.001$). The findings align with those reported by Altop et al. (2019). In general, there was an increase in the levels of crude protein and crude fat. The nutrient composition of feedstuffs is enhanced by the application of the solid culture fermentation method (Wang et al., 2017; Altop et al., 2018). In a study conducted by Li et al. (2020), the crude protein level of soybean meal fermented with *L. acidophilus* was observed to increase, a result that is consistent with the findings presented here. This increase can be attributed to microbial protein production. The observed increase in crude cellulose in the fermented samples can be attributed to alterations in the nutrient composition of the feedstuff. In a study conducted by Güngör et al. (2017), it was reported that the fermentation of cherry pits with different subspecies of microorganisms resulted in varying impacts on HS levels. It is possible that there was a relative increase in crude cellulose rates due to a lack of microorganism-substrate compatibility or a decrease in other nutrients (carbohydrates).

CONCLUSION

Fermentation has been demonstrated to positively impact the nutrient composition of various agricultural waste materials, including black carrot pulp. The optimal fermentation period for black carrot pulp with *L. acidophilus* was three days without enzyme.

REFERENCES

Adeyemi, O. A., Sobayo, R. A., Aluko, F. A., & Oke, D. B. 2008. Utilization of rumen filtrate fermented corn-cobs by weaner rabbits. Nigerian Journal of Animal Production, 35(1), 69-75.

Ahmad, T.; Cawood, M.; Iqbal, Q.; Ariño, A.; Batool, A.; Sabir Tariq, R.M.; Azam, M.; Akhtar, S. Phytochemicals in daucus carota and their health benefits—Review article. Foods 2019, 8, 424.

Akinfemi, A. (2010). Nutritive value and in vitro gas production of fungal treated maize cobs. African Journal of Food, Agriculture, Nutrition and Development, 10(8).

Akyıldız A, Yemler Bilgisi Laboratuvar Kılavuzu. Ankara. Ankara Üniversitesi Ziraat Fakültesi Yayınları, 1984.

Akyıldız, A. (1984). *Yemler bilgisi laboratuvar kılavuzu.* Ankara: Ankara Üniversitesi Ziraat Fakültesi Yayınları.

Altop, A., Coskun, I., Filik, G., Kucukgul, A., Bekiroglu, Y. G., Cayan, H., & Gungor, E. (2018). Amino acid, mineral, condensed tannin, and other chemical contents of olive leaves (Olea europaea L.) processed via solid-state fermentation using selected Aspergillus niger strains.

Altop, A., Güngör, E., & Erener, G. (2021). Different Aspergillus niger Strains and Inoculum Levels Affect the Nutritional Composition of Olive Leaves in Solid-state Fermentation. *Turkish Journal of Agriculture-Food Science and Technology*, *9*(12), 2303-2307.

Ari, M. M., & Ayanwale, B. A. 2012. Nutrient retention and serum profile of broilers fed fermented African Locust beans (Parkia filicoide). Asian Journal of Agricultural Research, 6(3); 129-136.

Ari, M. M., Ayanwale, B. A., Adama, T. Z., & Olatunji, E. A. 2012. Fermentation Technology and Bioengineering.

Assis AJ, Campos JMS, Filho S, Queiroz AC, Lana R, Euclydes RF, et al. Citrus pulp in diets for milking cows. 1. Intake of nutrients, milk production and composition. Revista Brasileira de Zootecnia, 2004;33:242–50.

Çenesiz, A.A., Ceylan, N., Çiftci, İ., Yavaş, İ., Taşkesen, O., Kıyak, O. ve Mueller, M. 2017. Rasyonlarında guanidino asetik asit ilavesinin büyüme performansı ve et kalitesi üzerine etkileri, 4. Uluslararası Beyaz Et Kongresi, 26-30 Nisan, Kongre Kitabı, 227-232, Antalya

Gajewski, M.; Szymczak, P.; Dabrowska, A.; Kret, A.; Elkner, K.; Danilcenko, H. Some aspects of nutritive and biological value of carrot cultivars with orange. yellow and purple-coloured roots. Veg. Crop. Res. Bull. 2007, 67, 149–161.

Güngör E, Erener G. Katı faz fermantasyonunun ceviz küspesinin besinsel kompozisyonu ve in vitro sindirilebilirliği üzerine etkisi. Balevi T, editör. Kanatlıların Beslenmelerinde Son Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2023. p.64-9.

Kamiloglu, S.; Van Camp, J.; Capanoglu, E. Black carrot polyphenols: Effect of processing. storage and digestion—An overview. Phytochem. Rev. 2018, 17, 379–395.

Mitchell, D. A., Krieger, N., Stuart, D. M., & Pandey, A. (2000). New developments in solid-state fermentation: II. Rational approaches to the design, operation and scale-up of bioreactors. *Process Biochemistry*, *35*(10), 1211-1225.

Nazok A, Rezaei M, Sayyahzadeh H. Effect of different levels of dried citrus pulp on performance, egg quality, and blood parameters of laying hens in early phase of production. Tropical Animal Health and Production 2010;42:737–42.

Özlü Ş, Altop A. Çeşitli probiyotik mikroorganizmalar tarafından fermente edilmiş zeytin yapraklarının besin değeri ve in vitro sindirilebilirliği. Balevi T, editör. Kanatlıların Beslenmelerinde Son Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2023. p.70-4.

Steudler, S., Werner, A., & Walther, T. (2019). It is the mix that matters: Substrate-specific enzyme production from filamentous fungi and bacteria through solid-state fermentation. *Solid State Fermentation: Research and Industrial Applications*, 51-81.

Wang JH, Guo H, Zhang TR, Wang H, Liu BN, Xiao S. Growth performance and digestion improvement of juvenile sea cucumber Apostichopus japonicus fed by solid-state fermentation diet. Aquaculture Nutrition. 2017;23(6):1312-1318.

Yoo, K.S.; Bang, H.; Pike, L.; Patil, B.S.; Lee, E.J. Comparing carotene. anthocyanins. and terpenoid concentrations in selected carrot lines of different colors. Hortic. Environ. Biotechnol. 2020, 61, 385–393

SOLID-STATE FERMENTATION OF SUNFLOWER HULLS: ENZYME EFFECTS ON NUTRIENT COMPOSITION

Şevket ÖZLÜ[1], Emrah GÜNGÖR[1], Bahar GÜNGÖR[2], Aydın ALTOP[1], Güray ERENER[1]

[1] Ondokuz Mayıs University, Faculty of Agriculture, Dept of Animal Science, Samsun, Turkey
[2] Ondokuz Mayıs University, Faculty of Science, Department of Molecular Biology and Genetics, Samsun, Turkey

Corresponding author e-mail: sevket.ozlu@omu.edu.tr

ABSTRACT

This study investigated the effects of exogenous enzymes on the nutrient composition of fermented sunflower seed hulls (SSH) in the solid-state fermentation method. The research was conducted in a 3 x 2 x 5 factorial experiment. Three different fermentation times (3-5 and 7 days) and two different enzyme additions (yes, no) were made in each replicate. Five replicates were conducted for each treatment group, with the total number of samples reaching 35, including the control group. Before fermentation, the SSH was ground to a particle size of 1 mm and added to the fermenter. The samples were sterilized at 121 °C for 15 minutes. The enzyme used in this study was obtained from a private company and comprises a combination of phytase, xylanase, and beta-glucanase. The enzyme was then added to a quantity of 1 g to each fermentation media and sterilized via UV light. *L. acidophilus* was incubated in a shaking incubator at 30 °C and 120 rpm for 48 hours using MRS broth. Then, 1 ml of the *L. acidophilus* culture (10^8 cfu/ml) was added to the sterilized fermenters. The research findings showed that the nutrient composition of SSH was improved through solid-state fermentation with enzymes. This enhancement led to an increase in crude protein levels and a notable decrease in crude fiber levels. Notably, a significant relationship between fermentation time, enzyme utilization, and all parameters was observed ($P < 0.001$). The sample fermented for five days with enzymes exhibited the highest crude protein and lowest crude fiber levels ($P < 0.001$). Conversely, the non-fermented sample had the highest crude fat levels ($P < 0.001$). . This study confirms increased crude protein and reduced crude fiber levels after fermentation. The ideal fermentation period for SSM with *L. acidophilus* is five days, supplemented with enzymes.
Key Words: Solid-state fermentation, sunflower seed hulls, *L. acidophilus*, enzyme

INTRODUCTION

The sunflower (*Helianthus annus* L.) is a plant that is cultivated under a variety of environmental conditions and utilized in the production of numerous products, including oil, meal, and biodiesel (Kallivroussis et al., 2002; Yegerov et al., 2019). One of the byproducts of these products is sunflower seed husk (SSH). It is utilized as agricultural waste in worldwide (Casoni et al., 2019). In Turkey, the husk is used as a solid fuel or as an alternative to straw for animal feeding.

The nutritional value of sunflower seed husk (SSH) is considerably lower than that of sunflower seed meal (SSM). The nutrient content of SSH varies according to the method of obtaining it, just like SSM (Golob et al., 2008). The crude protein level is between 4 and 8 percent, while the crude fiber content is between 50 and 55 percent (Cancalon, 1971). Although these fiber levels represent an alternative feedstuff for ruminant animals, their low protein content limits their use. Furthermore, its use in poultry nutrition is also very limited (Kasaeizadeh et al., 2024). Given these considerations, it is important to identify and implement strategies for utilizing such agricultural byproducts in animal nutrition, particularly in light of their environmental impact (Xie et al., 2016). One such method is solid-state fermentation (Mitchell et al., 2000).

The term "solid-state fermentation" refers to the biological and chemical activities of microorganisms on various substrates under suitable environmental conditions (temperature, pH, humidity, etc.) (Steudler et al., 2019). The microorganisms used are typically probiotic, which confers advantages to raw materials in numerous ways (Adeyemi et al., 2008; Akinfemi, 2010; Ari & Ayanwale, 2012; Ari et al., 2012; Güngör & Erener, 2023; Özlü & Altop, 2023). Consequently, solid-state fermentation enables the recycling of raw materials classified as waste and possessing nutritional properties (Altop et al., 2022). This study aimed to investigate the effects of exogenous enzymes on the nutrient composition of fermented SSH in the solid-state fermentation method.

MATERIAL AND METHOD

The study conducted a 3 x 2 x 5 factorial experiment involving three fermentation periods - 3, 5, and 7 days. Each period had two enzyme addition options: yes or no. For each treatment group, there were five replicates, giving a total of 35 samples, including the control group. Refer to Table 1 for the experimental layout.

Table 1. Plan of the research

CONTROL	Enzyme -/1	Enzyme -/2	Enzyme -/3	Enzyme -/4	Enzyme -/5
3 Days	Enzyme +/3-1	Enzyme +/3-2	Enzyme +/3-3	Enzyme +/3-4	Enzyme +/3-5
	Enzyme -/3-1	Enzyme -/3-2	Enzyme -/3-3	Enzyme -/3-4	Enzyme -/3-5
5 Days	Enzyme +/5-1	Enzyme +/5-2	Enzyme +/5-3	Enzyme +/5-4	Enzyme +/5-5
	Enzyme -/5-1	Enzyme -/5-2	Enzyme -/5-3	Enzyme -/5-4	Enzyme -/5-5
7 Days	Enzyme +/7-1	Enzyme +/7-2	Enzyme +/7-3	Enzyme +/7-4	Enzyme +/7-5
	Enzyme -/7-1	Enzyme -/7-2	Enzyme -/7-3	Enzyme -/7-4	Enzyme -/7-5

SSH, sourced locally, was dried at 75°C and stored at 4°C until the study. *L. acidophilus* (ATCC 4356) was procured from the American Type Culture Collection (ATCC) as the microbial inoculant.

Prior to fermentation, the SSH was ground to a 1 mm size and placed in the fermenter, along with 100 g of the pulp and 500 ml of distilled water in each. Microbial growth was facilitated by adding 85.5 g of nutritional salt to 1 lt of distilled water. After sterilization at 121°C for 15 minutes, a blend of phytase, xylanase, and beta-glucanase enzymes from a commercial source was added to each fermentation medium and sterilized using UV light.

The *L. acidophilus* culture (at 108 cfu/ml) was incubated in MRS broth at 30°C and 120 rpm for 48 hours. Subsequently, 1 ml of the culture was added to the sterilized fermenters. The inoculated samples underwent fermentation at 30°C for 3-5-7 days. Post-fermentation, analysis for dry matter, ash, crude protein, crude fiber, and ether extract was carried out as per Akyıldız (1984).

Data from the study underwent analysis of variance utilizing the GLM ANOVA/MANOVA function in Statistica (1984) with a 3 x 2 factorial setup representing the three fermentation periods, two enzyme adoptions, and a randomized-block design. Upon establishing statistical significance ($P < 0.05$) among the groups, Tukey's HSD test identified the specific group with significant differences ($P = 0.05$).

RESULTS AND DISCUSSION

The research findings showed that the nutrient composition of SSH was improved through solid-state fermentation with enzymes. This enhancement led to an increase in crude protein levels and a notable decrease in crude fiber levels. Notably, a significant relationship between fermentation time, enzyme utilization, and all parameters was observed ($P < 0.001$). The sample fermented for five days with enzymes exhibited the highest crude protein and lowest crude fiber levels ($P < 0.001$). Conversely, the non-fermented sample had the highest crude fat levels ($P < 0.001$). Additionally, Chi and Cho (2016) noted a reduction in ether extract content in soybean meal fermented with *L. acidophilus*.

The main goal of fermentation studies on animal feeds is to lower crude fiber levels and boost crude protein levels (Wu et al., 2015). The rise in crude protein content is likely due to microbial protein production by microorganisms (Özlü & Altop, 2023). These results align with those of Hajimohammadi et al. (2020), who fermented sesame meal with S. cerevisiae and *L. acidophilus*, significantly increasing crude protein levels. Crude fiber components are structural carbohydrates in plant cell walls and are abundant in agricultural residues (Graminha et al., 2008). Crude fiber comprises cellulose, hemicellulose, lignin, pectin, and xylan compounds (Özlü & Altop, 2023). Decreasing these compounds enhances feed digestibility. Reducing crude fiber content in solid-state fermentation is achievable through enzymes like cellulase, hemicellulase, and β-glucosidase (Wu et al., 2015). Enzymes and microorganisms achieve this reduction (Chuang et al., 2019; Maiorano et al., 2022).

CONCLUSION

Fermentation enhances the nutrient composition of agricultural wastes like SSM, promoting better feed utilization and offering probiotic benefits. This study confirms increased crude protein and reduced crude fiber levels after fermentation. The ideal fermentation period for SSM with *L. acidophilus* is five days, supplemented with enzymes.

REFERENCES

Adeyemi, O. A., Sobayo, R. A., Aluko, F. A., & Oke, D. B. 2008. Utilization of rumen filtrate fermented corn-cobs by weaner rabbits. Nigerian Journal of Animal Production, 35(1), 69-75.

Akinfemi, A. (2010). Nutritive value and in vitro gas production of fungal treated maize cobs. African Journal of Food, Agriculture, Nutrition and Development, 10(8).

Akyıldız A, Yemler Bilgisi Laboratuvar Kılavuzu. Ankara. Ankara Üniversitesi Ziraat Fakültesi Yayınları, 1984.

Altop, A., Erener, G., Güngör, E., Özlü, Ş., Güngör, B., (2022). Katı Kültür Fermantasyonuyla Zeytin Yaprağının Kanatlı Hayvan Beslemede Antimikrobiyal Etkili Yem Katkı Maddesine Dönüştürülme Potansiyelinin Araştırılması. OMÜ BAP PYO.ZRT.1908.22.004 Numaralı Proje Sonuç Raporu.

Altop, A., Erener, G., Güngör, E., Özlü, Ş., Güngör, B., (2022). Katı Kültür Fermantasyonuyla Zeytin Yaprağının Kanatlı Hayvan Beslemede Antimikrobiyal Etkili Yem Katkı Maddesine Dönüştürülme Potansiyelinin Araştırılması. OMÜ BAP PYO.ZRT.1908.22.004 Numaralı Proje Sonuç Raporu.

Ari, M. M., & Ayanwale, B. A. 2012. Nutrient retention and serum profile of broilers fed fermented African Locust beans (Parkia filicoide). Asian Journal of Agricultural Research, 6(3); 129-136.

Ari, M. M., Ayanwale, B. A., Adama, T. Z., & Olatunji, E. A. 2012. Fermentation Technology and Bioengineering.

Cancalon, P. (1971). Chemical composition of sunflower seed hulls. *Journal of the American Oil Chemists' Society*, *48*(10), 629.

Casoni, A. I., Gutierrez, V. S., & Volpe, M. A. (2019). Conversion of sunflower seed hulls, waste from edible oil production, into valuable products. *Journal of Environmental Chemical Engineering*, *7*(1), 102893.

Chi CH, Cho SJ. Improvement of bioactivity of soybean meal by solid-state fermentation with *Bacillus amyloliquefaciens* versus *Lactobacillus* spp. and *Saccharomyces cerevisiae*. LWT-Food Science and Technology. 2016;68:619-25.

Chuang WY, Lin WC, Hsieh YC, Huang CM, Chang SC, Lee TT. Evaluation of the combined use of *Saccharomyces cerevisiae* and *Aspergillus oryzae* with phytase fermentation products on growth, inflammatory, and intestinal morphology in broilers. Animals. 2019;9(12):1051.

Golob, P., Farrell, G., ve Orchard, J.E. 2008. "Crop Post-Harvest, Science and Technology, Volume 1, Principles and Practice", 1(2008), John Wiley & Sons.

Graminha E, Gonçalves A, Pirota R, Balsalobre M, Da Silva R, Gomes E. Enzyme production by solid-state fermentation: Application to animal nutrition. Animal Feed Science and Technology. 2008;144(1-2):1-22.

Güngör E, Erener G. Katı faz fermantasyonunun ceviz küspesinin besinsel kompozisyonu ve in vitro sindirilebilirliği üzerine etkisi. Balevi T, editör. Kanatlıların Beslenmelerinde Son Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2023. p.64-9.

Hajimohammadi A, Mottaghitalab M, Hashemi M. Effects of microbial fermented sesame meal and enzyme supplementation on the intestinal morphology, microbiota, pH, tibia bone and blood parameters of broiler chicks. Italian Journal of Animal Science. 2020;19(1):457-67.

Kallivroussis, L., Natsis, A., & Papadakis, G. (2002). RD—rural development: the energy balance of sunflower production for biodiesel in Greece. *Biosystems Engineering*, *81*(3), 347-354.

Kasaeizadeh, R., Salari, S., Abdollahi, M. R., & Baghban, F. (2024). The effect of particle size of sunflower hulls in diets containing different fat sources on growth performance, apparent ileal digestibility of nutrients and cecal microbial population of broiler chickens. *Animal Production*, *26*(1), 45-60.

Maiorano G, Ramires FA, Durante M, Palamà IE, Blando F, De Rinaldis G. The controlled semi-solid fermentation of seaweeds as a strategy for their stabilization and new food applications. Foods. 2022;11(18):2811.

Mitchell, D. A., Krieger, N., Stuart, D. M., & Pandey, A. (2000). New developments in solid-state fermentation: II. Rational approaches to the design, operation and scale-up of bioreactors. *Process Biochemistry*, *35*(10), 1211-1225.

Özlü Ş, Altop A. Çeşitli probiyotik mikroorganizmalar tarafından fermente edilmiş zeytin yapraklarının besin değeri ve in vitro sindirilebilirliği. Balevi T, editör. Kanatlıların Beslenmelerinde Son Gelişmeler. 1. Baskı. Ankara: Türkiye Klinikleri; 2023. p.70-4.

Steudler, S., Werner, A., & Walther, T. (2019). It is the mix that matters: Substrate-specific enzyme production from filamentous fungi and bacteria through solid-state fermentation. Solid State Fermentation: Research and Industrial Applications, 51-81.

Xie, P., Huang, L., Zhang, C. ve Zhang, Y.-l. (2016). Nutrient assessment of olive leaf residues processed by solid-state fermentation as an innovative feedstuff additive. Journal of Applied Microbiology. *121 (1). 28-40.*

Wu Q, Wang Z, Wang G, Li Y, Qi Y. Effects of feed supplemented with fermented pine needles (*Pinus ponderosa*) on growth performance and antioxidant status in broilers. Poultry Science. 2015;94(6):1138-44.

Yegorov, B., Turpurova, T., Sharabaeva, E., & Bondar, Y. (2019). Prospects of using by-products of sunflower oil production in compound feed industry. Journal of Food Science Technology Ukraine, 13, 106– 113. https://doi.org/10.15673/fst.v13i1.1337

DESIGN AND IMPLEMENTATION OF AN AUTONOMOUS PC POWER MANAGEMENT SYSTEM

Mustafa ÇETİNGÖZ

Trakya University, Institute of Natural Sciences, Department of Computer Engineering, Edirne, Türkiye

Corresponding author e-mail: mustafacetingoz@trakya.edu.tr

ABSTRACT

This paper presents a Autonomous Power Management System designed to reduce the energy consumption of PCs by automating the process of switching them on and off. The system comprises an access point (AP), a PC, and a mobile application. When a user enters the range of the AP, the mobile app automatically connects to it and sends a Wake-on-LAN (WoL) packet to wake up the PC. Upon powering on, a PC agent begins running, enabling smooth communication between the PC and the user's smartphone. When the user departs and communication drops, the PC agent initiates a sleep process after a predefined timeout period. This system operates autonomously, without user intervention, and contributes to significant energy savings.

Keywords: Power management, Energy saving, Wake-on-LAN, PC agent, Mobile application

INTRODUCTION

The global demand for electricity has risen sharply due to factors such as population growth, urbanization, and the widespread use of electronic devices, raising concerns about energy security, environmental sustainability, and rising costs. Increased energy consumption has negatively impacted the environment through air and water pollution, greenhouse gas emissions, and climate change. As a result, many countries have adopted energy conservation and efficiency strategies as key policy objectives to reduce environmental harm and improve energy security (Otsuka, 2023). Governments are promoting energy-efficient technologies to encourage reduced consumption and lower greenhouse gas emissions (An et al., 2016). In this context, energy efficiency—defined as reducing energy use without compromising productivity—plays a crucial role in lowering energy bills, reducing grid strain, and protecting the environment. Technological advancements, such as smart energy management systems, provide significant opportunities to enhance efficiency, reduce costs, and promote sustainability (Pandiyan et al., 2023).

Various strategies have been proposed to reduce energy consumption, including the development of Home Energy Management Systems (HEMS), which allow users to optimize energy use in residential settings. HEMS help customers adjust energy consumption to reduce utility costs and enhance energy efficiency, while maintaining comfort and convenience (Liu et al., 2022). These systems manage electrical appliances based on variables such as energy costs, load profiles, and environmental considerations, scheduling device usage to minimize consumption during peak demand periods (Ben

Slama & Mahmoud, 2023). Smart plugs, a key component in these systems, offer a practical solution for monitoring and controlling appliance energy use. These devices can be remotely managed via smartphone apps or voice-activated assistants, providing a simple means to reduce energy waste (Shakeri et al., 2020). A key benefit of using a smart plug is switching off appliances when on stand-by or unused, thus reducing energy consumption.

Numerous studies in the literature have explored smart home energy management systems and smart plugs. Horvat et al. proposed a BLE-enabled smart plug solution to control household devices and monitor energy consumption via mobile devices, eliminating the need for a gateway (Horvat et al., 2015). Thongkhao et al. introduced a Wi-Fi-based smart plug system controlled through a web application, demonstrating high accuracy in energy measurement for reliable management (Thongkhao & Pora, 2016). In an office environment, Choi et al. utilized BLE beacons to detect user presence, automatically switching smart plugs on or off to manage energy consumption remotely. However, the system faced challenges with smartphone battery drain (Choi et al., 2015).

There have been a number of studies on the subject of PC power control over IP networks. The study introduces EnergySave, a sophisticated energy-saving system that utilizes the established Wake-on-LAN (WoL) protocol to enable the remote activation of PCs from a centralized management server (Ricciardi et al., 2013). Upon registration of a client PC, its IP and MAC addresses are stored on the server, facilitating secure and effective power management for both local and remote IP-based computer networks. The system's capabilities are further enhanced by the development of a mathematical model for energy savings, which allows for simulation-based assessments of the potential energy reductions achievable through its implementation.

This study (Byon et al., 2015) introduces a model of suspended mode combined with remote wake-up functionality aimed at reducing idle power consumption in PCs. This model is centered around a system architecture that includes components such as a Wakeup Server, network infrastructure, and PCs. The Wakeup Server plays a critical role by gathering detailed state information from connected PCs and sending Wake-on-LAN messages to initiate the wake-up process when necessary. The network device, operating at layer 2, is responsible for forwarding these WoL messages from the server to the respective PCs. Users can remotely interact with their PCs through the Wakeup Server, which enables them to wake up their devices from a suspended state and subsequently use them after the wake-up process is completed.

Polisave, as described in (Chiaraviglio & Mellia, 2010), is a client-server-based system that allows users to schedule the power states of their PCs, providing remote control over turning them on or off. The system provides users with the ability to power their PCs on or off interactively from a distant location. The client-side application installed on the PC periodically transmits information such as the IP address and MAC address to the server. The server then aggregates the status data from multiple PCs and responds with a message that specifies an action—such as hibernate, standby, power-off, or wait—along with the reporting interval. The shutdown process is initiated based on the server's instructions.

In this study, we developed an Autonomous Power Management System for PCs aimed at enhancing convenience, improving efficiency, and reducing energy consumption by automating the power control of PCs. The system consists of two key components: a smartphone application and a PC agent. The smartphone application scans for available Wi-Fi SSIDs, establishes a connection upon detection, and transmits a Wake-on-LAN packet to initiate the PC's power-on process. The PC agent enables communication between the PC and the smartphone application. Not only does this system save time by providing automatic power control without the need for an additional device, but it also reduces energy consumption by automatically turning off the PC when the user leaves the vicinity.

In the following section, a comprehensive overview of the Materials and Methods is provided, detailing the Wake-on-LAN network protocol that plays a critical role in the proposed system. This section also outlines the architectural components of the system. The subsequent section presents the experimental study and its outcomes. Finally, the paper concludes with a summary of key findings and a discussion of the system's implications.

MATERIAL AND METHOD

This section introduces the Wake-on-LAN network protocol, a key element in the proposed system for remotely powering on PCs. Additionally, the proposed system architecture is presented, which includes a mobile application and a PC agent, both working together to facilitate seamless control and communication between the user's smartphone and their PC.

2.1. Wake-on-LAN

To reduce PC power consumption, both active and idle time management strategies are applied. During low activity periods, methods such as lowering CPU voltage and clock frequency are employed. Additionally, operating systems like Linux and Windows use suspended modes to minimize idle time consumption. When a PC exceeds a defined idle period, it transitions to suspended mode, where key components, including the CPU and graphics card, are powered down, significantly reducing energy use. The two primary suspended states are "suspend to RAM" and "suspend to disk." In suspend-to-RAM, execution data is stored in memory, while most hardware components are powered off. In suspend-to-disk, data is saved to the disk. However, during suspension, PCs cannot respond to external network requests, requiring remote wake-up methods like Wake-on-LAN to ensure they can be reactivated when needed.

Wake-on-LAN is a network protocol that facilitates the remote activation of a personal computer. This feature allows a computer in a low-power state, such as sleep or hibernate, to be powered on via a network signal, eliminating the need for manual interaction. WoL operates through the computer's network interface card (NIC) and requires appropriate configuration within the motherboard's BIOS settings. When connected to a local area network (LAN) with WoL enabled, the NIC remains in a standby mode, continuously awaiting a "magic packet." This packet, containing the computer's unique Media Access Control (MAC) address, is sent by another device on the same network. Once the correct magic packet is received, the targeted computer is triggered to power on. Although the packet is broadcast to all devices on the network, only the machine with the corresponding MAC address will respond to the signal, initiating the power-up process.

2.2. Proposed System Archıtecture

This section outlines the architecture of the proposed Autonomous Power Management System for PCs, consisting of two primary components: a mobile application and a PC agent. The main architecture of the whole system is shown in Figure 1 and is designed to optimize the power management of computers in a home or office environment.

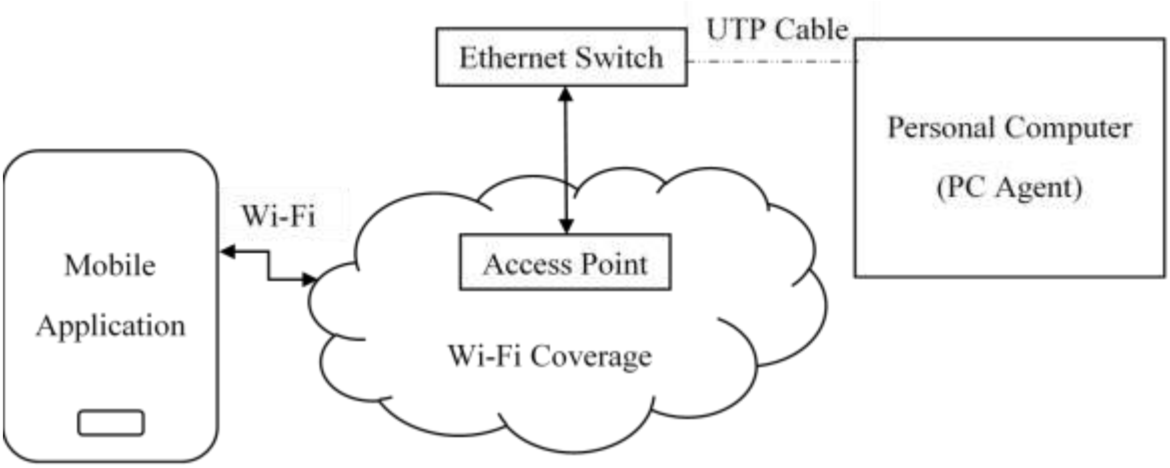


Figure 1. Architecture of autonomous PC power management system

2.2.1 Mobile application

Most modern smartphones support Wi-Fi technology and almost which is designed to drastically minimize battery usage during wireless communication. The mobile application developed in this study operates by scanning available Wi-Fi SSIDs in the background. Upon detecting a preconfigured SSID, the application attempts to establish a connection with the access point (AP). Once successfully connected, the app transmits a WoL packet to the target PC. Users have two options within the mobile app: to automatically initiate the power-on process or to power on the PC following user approval. The WoL packet includes critical network information, such as the IP address, MAC address, and subnet mask of the PC. Upon receiving the WoL packet, the PC initiates the power-on process and prepares to activate the PC Agent. Following the PC's power-up, the PC Agent is automatically launched and begins awaiting periodic heartbeat signals from the mobile application.

2.2.2 PC Agent

Personal computers (PCs) represent a significant proportion of IT devices in both office and home environments and are essential for users' daily tasks and overall productivity. In office settings, PCs and monitors offer substantial opportunities for energy conservation (Choi et al., 2015). The developed PC Agent operates in the background, continuously awaiting periodic heartbeat signals from the mobile application to confirm the device's active state. Upon receiving these signals, the agent acknowledges that the PC is functioning and communicates this status back to the mobile application.

The PC Agent monitors the regular receipt of heartbeat signals from the mobile app. In scenarios where the mobile app exits the Wi-Fi coverage area or disconnects from the network, the heartbeat timeout value increases incrementally. Once this value exceeds a predefined threshold, the PC Agent initiates the power-saving procedure, either by putting the system into sleep mode or triggering the hibernation process. This mechanism ensures energy efficiency by automatically reducing power consumption when the user is no longer in proximity. Main screen of PC Agent software is shown in Figure 2.

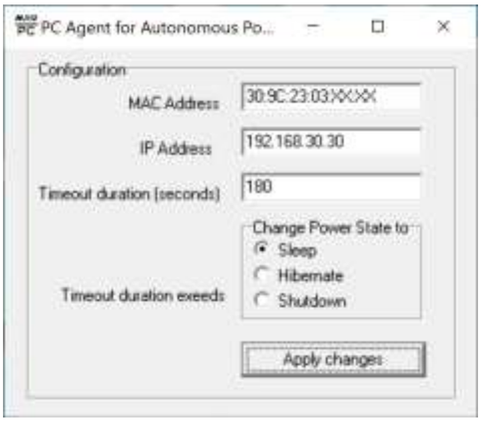


Figure 2. Main screen of PC Agent

EXPERIMENTAL STUDY AND RESULTS

The experimental study was conducted in a real-world office environment, involving five employees, each utilizing a personal computer (PC) for daily tasks. The office infrastructure featured an access point (AP) that provided Wi-Fi connectivity for mobile devices, while the PCs were connected to the network via UTP cables to ensure stable and fast data transmission. To facilitate the energy-saving mechanism, a PC Agent software was installed on each PC. Once the PC agent software had been installed, the PC's MAC and IP address information was recorded for input into the mobile application.

For mobile device integration, the mobile application was installed on each employee's smartphone. The app settings were configured using key details such as the Wi-Fi SSID, username, password, and the respective PC's MAC and IP addresses. Employees were instructed to run the mobile application continuously in the background. This mobile application ensured the smooth operation of the proposed autonomous power management system by communicating with the access point to detect the presence of the user and trigger PC power states accordingly.

The implemented system resulted in a fully automated process for managing the PCs' power states. When employees arrived at the office and their smartphones connected to the designated Wi-Fi network, the mobile application detected their presence and automatically sent a Wake-on-LAN packet, powering on the associated PCs. Conversely, when employees left the office, the application recognized the departure and initiated a shutdown process for the PCs, reducing idle power consumption and enhancing overall energy efficiency. This automated system demonstrated significant potential in optimizing energy usage in office environments.

CONCLUSIONS

This study introduces an Autonomous Power Management System aimed at minimizing PC energy consumption through automated control of power states. The system effectively integrates a mobile application, an access point (AP), and a PC, leveraging Wake-on-LAN technology to remotely activate PCs as users enter the AP's vicinity. Once activated, the PC agent facilitates seamless communication between the PC and the user's mobile device. When the user departs and communication ceases, the system autonomously transitions the PC into sleep mode after a preset period. This self-sustaining solution not only eliminates the need for manual intervention but also offers substantial energy savings by optimizing power states based on user presence.

REFERENCES

An, A. M., Zheng, B. R., Zheng, H. C., Zheng, C. D., & Du, P. D. (2016). Benefit Analysis and Evaluation of Distributed Generation in Distribution Network under Active Management. Proceedings of the 28th Chinese Control and Decision Conference (2016 Ccdc), 6031-6035. <Go to ISI>://WOS:000383222306053

Ben Slama, S., & Mahmoud, M. (2023). A deep learning model for intelligent home energy management system using renewable. Engineering Applications of Artificial Intelligence, 123. https://doi.org/ARTN 106388

10.1016/j.engappai.2023.106388

Byon, S., Kwon, E., Jung, E. S., Lee, Y. T., & Ryu, W. (2015, 28-30 Oct. 2015). An implementation of PC state management in IP networks. 2015 International Conference on Information and Communication Technology Convergence (ICTC),

Chiaraviglio, L., & Mellia, M. (2010, 23-25 Sept. 2010). PoliSave: Efficient power management of Campus PCs. SoftCOM 2010, 18th International Conference on Software, Telecommunications and Computer Networks,

Choi, M., Park, W. K., & Lee, I. (2015). Smart Office Energy-Saving Service Using Bluetooth Low Energy Beacons and Smart Plugs. 2015 Ieee International Conference on Data Science and Data Intensive Systems, 247-251. https://doi.org/10.1109/Dsdis.2015.69

Horvat, I., Lukac, N., Pavlovic, R., & Starcevic, D. (2015). Smart Plug solution based on Bluetooth Low Energy. 2015 Ieee 5th International Conference on Consumer Electronics - Berlin (Icce-Berlin), 435-437. <Go to ISI>://WOS:000380452700095

Liu, Y. Y., Ma, J., Xing, X. J., Liu, X. L., & Wang, W. (2022). A home energy management system incorporating data-driven uncertainty-aware user preference. Applied Energy, 326. https://doi.org/ARTN 119911

10.1016/j.apenergy.2022.119911

Otsuka, A. (2023). Industrial electricity consumption efficiency and energy policy in Japan. Utilities Policy, 81. https://doi.org/ARTN 101519

10.1016/j.jup.2023.101519

Pandiyan, P., Saravanan, S., Usha, K., Kannadasan, R., Alsharif, M. H., & Kim, M. K. (2023). Technological advancements toward smart energy management in smart cities. Energy Reports, 10, 648-677. https://doi.org/10.1016/j.egyr.2023.07.021

Ricciardi, S., Santos-Boada, G., Careglio, D., Palmieri, F., & Fiore, U. (2013, 28-31 May 2013). Evaluating energy savings in WoL-enabled networks of PCs. 2013 IEEE International Symposium on Industrial Electronics,

Shakeri, M., Pasupuleti, J., Amin, N., Rokonuzzaman, M., Low, F. W., Yaw, C. T., Asim, N., Samsudin, N. A., Tiong, S. K., Hen, C. K., & Lai, C. W. (2020). An Overview of the Building Energy Management System Considering the Demand Response Programs, Smart Strategies and Smart Grid. Energies, 13(13). https://doi.org/ARTN 3299

10.3390/en13133299

Thongkhao, Y., & Pora, W. (2016). A Low-cost Wi-Fi Smart Plug with On-off and Energy Metering Functions. 2016 13th International Conference on Electrical Engineering/Electronics, Computer, Telecommunications and Information Technology (Ecti-Con). <Go to ISI>://WOS:000389506000042

A BLE-BASED MONITORING SYSTEM FOR WHEAT STORAGE

Mustafa ÇETİNGÖZ

Trakya University, Institute of Natural Sciences, Department of Computer Engineering, Edirne, Türkiye

Corresponding author e-mail: mustafacetingoz@trakya.edu.tr

ABSTRACT

In agricultural practices, wheats are sometimes stored in warehouses situated on farms or near residential areas. However, excessive moisture or water leakage within these storage environments can lead to the deterioration and mold growth of stored wheats, presenting significant challenges for their long-term preservation. If such issues are not promptly detected, they can result in severe degradation of wheat quality. This paper introduces the design and implementation of a temperature, humidity and CO2 control system to address these risks. The system consists of two primary components: Bluetooth Low Energy (BLE)-based sensor module and a mobile application. The sensor module periodically transmits temperature and humidity data, and owing to the low energy consumption of BLE technology, the module can function on battery power for more than one year. The mobile application receives the BLE broadcasts and stores the collected data. In the event of elevated moisture or temperature levels during storage, the application alerts the user, enabling timely corrective actions.

Keywords: Temperature control, Wheat storage, Humidity detection, BLE in agriculture, Mobile application

INTRODUCTION

Agriculture remains a cornerstone of many national economies, serving as a primary source of income for a significant portion of the population. In several countries, a substantial percentage of the economy is driven by agricultural activities, with wheat being one of the most essential crops. Wheat's versatility, used in producing bread, pasta, spaghetti, and numerous other food items, makes it one of the most widely consumed grains globally. Despite this, a significant portion of agricultural produce, including wheat, fails to reach consumers due to damage incurred during farming, transportation, or storage. One of the most critical challenges in maintaining wheat grain quality is the inability of farmers to detect early signs of moisture or fungal contamination, which promotes mold growth and leads to gradual spoilage. Beyond moisture and mold, pests such as worms and fungi also contribute to grain degradation, reducing the storage life of wheat. These issues often result in shortages of wheat-based products, leading to increased market prices and economic losses, which underscore the importance of effective monitoring systems for wheat storage.

Several storage methods, such as bags, warehouses, and silos, are used by both individuals and larger organizations to store grains. However, these solutions can be cost-prohibitive for small-scale producers and farmers. Many farmers opt to store their wheat in warehouses located on farms or near residential areas, often lacking the specialized knowledge required to establish optimal storage conditions. Effective monitoring and management of these grains are crucial to ensuring their longevity and reducing post-harvest losses. Therefore, improving storage supervision through better understanding and research is critical to addressing the ongoing global issue of grain loss after harvest.

The Internet of Things (IoT) refers to a network of interconnected objects that communicate wirelessly without requiring human intervention. IoT has found widespread application in agriculture, with precision farming being a key example of its significant impact on the industry (Dholu & Ghodinde, 2018). The development of IoT-based systems integrated with smartphone applications has proven to be an efficient and practical approach for addressing grain storage monitoring challenges. Beyond grain storage, IoT applications are utilized across various sectors, including indoor environmental monitoring (Assante & Fornaro, 2019), healthcare (Abdulmalek et al., 2022), traffic management (Soni & Saraswat, 2017), smart buildings (Jia et al., 2019), and smart cities (Brincat et al., 2019).

In agriculture, Bluetooth Low Energy (BLE), a key IoT technology, plays a critical role due to its low energy consumption, and there are many IoT-based studies in the literature. In (Kodali et al., 2020), a smart monitoring system was developed to enhance food storage and reduce wastage by using IoT technology. The system, detailed in the paper, integrates a microcontroller with various sensors to track temperature, humidity, and food quality across multiple stages, including the farm, processing plants, warehouses, and markets. Additionally, the system actively moderates the storage environment by controlling fans and cooling units to maintain optimal conditions. By following a first-in, first-out strategy, the system also minimizes food grain expiration and waste, thereby improving efficiency and sustainability in the food supply chain.

A study introduced an intelligent warehousing system that leverages RFID technology for automated data collection across various processes, including goods arrival, inbound and outbound deliveries, and transfers (Yang, 2019). This system enhances efficiency by integrating with ERP software, enabling real-time data management and reducing operational costs. A study focused on the safe storage of cereal grains, particularly for long-duration space missions, by addressing the risks of moisture-induced spoilage. It introduced a printed circuit board (PCB) capacitive fringing field sensor to detect moisture imbibed by corn kernels, as moisture can lead to germination, spoilage, or microorganism growth (Dean et al., 2019).

This paper outlines the design and implementation of a BLE-enabled smart grain storage monitoring system using an ESP32 microcontroller connected to a temperature and humidity sensor (DHT11) and a carbon dioxide sensor (MQ-135). The ESP32 microcontroller serves as the core of the system, broadcasting data through BLE. The second critical component is a smartphone application that receives these BLE broadcasts and alerts the user if any environmental data exceed predefined thresholds.

The following section of this paper addresses the materials and methods, with a focus on BLE technology, the ESP32 microcontroller, and the sensors utilized for monitoring humidity, temperature, and CO2. The subsequent section covers the experimental study.

MATERIAL AND METHOD

This section introduces Bluetooth Low Energy (BLE), a fundamental technology in the proposed system, designed for efficient data transmission with minimal power consumption. Additionally, it presents the key component of the system architecture, the ESP32 microcontroller, which functions as the core of the sensor module.

2.1. Bluetooth Low Energy Technology

Bluetooth Low Energy (BLE) is a wireless communication technology engineered to prioritize low power consumption, making it particularly suited for battery-operated devices. In contrast to other wireless technologies such as Wi-Fi and ZigBee, BLE's lower energy usage makes it ideal for applications in which power efficiency is critical. Although Wi-Fi typically offers a longer range, the coverage provided by BLE is adequate for many applications, especially in confined spaces like homes, offices, and other indoor environments. Smartwatches, PCs, tablets, smartphones, and other Internet of Things devices are just a few of the devices that use BLE technology. Low power consumption, low cost, and the use of simpler radio transceiver circuits are given top priority in the design of BLE technology (Shahzad & Oelmann, 2014).

Data and advertising are the two main types of radio frequency channels used by BLE (Taskin et al., 2018). Channels 0 to 36 are used for data transmission, while channels 37, 38, and 39 serve as advertising channels, which are employed for discovering devices, broadcasting data, and initiating connections (de Cerio et al., 2017). A key feature of BLE technology is its ability to transmit data via advertising without the need for establishing a dedicated peer-to-peer connection. These advertising packets, typically short and concise, enable rapid transmission while minimizing energy consumption. Adjusting the advertising intervals allows for a trade-off between power consumption and update frequency: shorter intervals provide more frequent updates at the cost of increased power consumption, while longer intervals conserve energy but reduce the update rate (Liu et al., 2013). Devices equipped with scanning capabilities can listen for these advertising packets and extract relevant information without requiring a formal connection, further enhancing BLE's efficiency in low-power environments.

2.2. ESP32 Microcontroller

The ESP32, developed by Espressif Systems and introduced in 2016, is a highly capable microcontroller that integrates both Wi-Fi and Bluetooth Low Energy (BLE) functionalities (Singh & Kapoor, 2017). Designed for use in mobile electronics, handheld devices, and Internet of Things (IoT) applications (Mishu et al., 2021), the ESP32 has become a popular choice for developers due to its versatility and power efficiency. Its wide range of low-power modes makes it particularly suitable for battery-operated devices, enabling developers to program the microcontroller to enter sleep modes and periodically wake up, thereby conserving energy. The ESP32 supports a broad array of peripheral interfaces, such as SPI, I2C, UART, and PWM, which allow for seamless integration with various sensors, displays, and external components.

2.3. Main Archıtecture of Wheat Monitoring System

The proposed monitoring system aims to continuously monitor environmental conditions within wheat storage facilities to safeguard grain quality and ensure safe storage. The system architecture is composed of two primary components: the sensor module and the mobile application. The sensor module is responsible for collecting environmental data such as temperature, humidity, and CO2 levels using integrated sensors. Meanwhile, the mobile application, compatible with BLE-enabled smartphones, serves as the user interface, receiving real-time data broadcasts from the sensor module. The system's overall architecture is depicted in Fig. 1, illustrating the interaction between the sensor module and the mobile app.

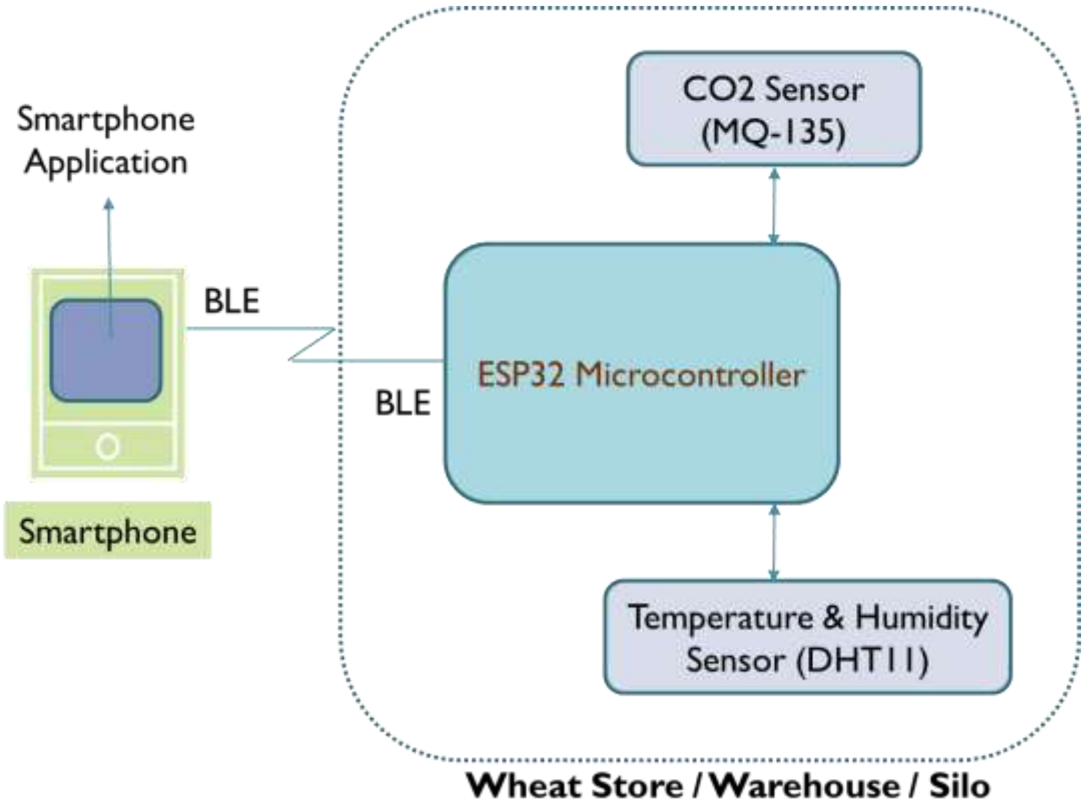


Figure 1. Architecture of wheat monitoring system

2.3.1 Sensor module

The core of the sensor module is the ESP32 microcontroller, which integrates a DHT11 sensor to measure temperature and humidity, alongside an MQ-135 sensor for detecting CO2 levels. These sensors enable the system to monitor environmental conditions that could potentially compromise the quality of stored wheat grain.

The DHT11 sensor, a low-cost solution for monitoring both humidity and temperature, has been integrated into the sensor module due to its ease of interfacing with the ESP32 microcontroller. The DHT11 operates within a temperature range of 0 to 50 degrees Celsius with an accuracy of ±2 degrees, while its humidity measurement capabilities cover a range of 20 to 80 percent relative humidity with a precision of ±5 percent. With a sampling rate of 1Hz, the sensor provides readings once per second, making it suitable for real-time monitoring in the proposed system.

Mold growth in stored grains can result in the emission of carbon dioxide (CO2), and similarly, pests such as rats, mice, and insects that consume the grains also release CO2 through respiration. To monitor such activity, the sensor module in the proposed system utilizes the MQ-135 sensor for CO2 detection. When elevated levels of CO2 are detected, indicating potential contamination or pest presence, the ESP32 microcontroller broadcasts this data wirelessly using Bluetooth Low Energy (BLE) technology, enabling real-time transmission to the user’s smartphone for prompt response. The BLE-based communication framework ensures efficient data transmission with minimal power consumption, making the system well-suited for long-term monitoring in storage environments.

2.3.2 Mobile application

The mobile application developed forms the other essential component of the proposed system architecture. Designed for BLE-enabled smartphones, the app receives

BLE advertising packets transmitted by the sensor modules, enabling continuous monitoring of environmental conditions. The app also incorporates a notification system that alerts users when temperature, humidity, or CO2 levels exceed predefined thresholds. If critical values are detected, the application sends an immediate alert to the user, ensuring timely action to safeguard the quality of the stored grain.

EXPERIMENTAL STUDY AND RESULTS

The prototype system was deployed in a wheat storage room located in a rural village, beneath the farmer's house. The farmer, who is typically occupied with daily activities such as cow feeding and other tasks in the garden, was provided with a smartphone that had the mobile application developed for this study installed. The farmer was instructed to keep the application running in the background to facilitate real-time data reception from the sensor module.

During the experiment, it was observed that the sensor module consistently transmitted environmental measurements to the smartphone via Bluetooth Low Energy (BLE). When the temperature and other measurement values exceeded the predefined threshold, the system successfully generated a notification on the mobile app, alerting the farmer to take appropriate action. This demonstrated the system's ability to provide timely alerts based on environmental conditions.

CONCLUSIONS

The proposed IoT-based system effectively addresses the challenge of monitoring and controlling environmental anomalies in wheat storage facilities. Using sensors to measure temperature, humidity and CO2 levels, the system enables early detection of conditions that could threaten grain quality. The integration of BLE technology allows for real-time data transmission, ensuring that storage conditions are continuously monitored and any abnormal variations are promptly detected.

A key feature of the system is the mobile application, which enhances user notifications by delivering real-time alerts when environmental conditions exceed predefined thresholds. This functionality allows users to take immediate corrective action, preventing spoilage and reducing losses in grain storage. The system's low power architecture and BLE connectivity make it ideal for grain monitoring applications. It provides an affordable and efficient solution for wheat storage monitoring. Future enhancements could focus on incorporating additional IoT sensors to expand the system's capabilities and further improve its response to abnormal situations.

REFERENCES

Abdulmalek, S., Nasir, A., Jabbar, W. A., Almuhaya, M. A. M., Bairagi, A. K., Khan, M. A.-M., & Kee, S.-H. (2022). IoT-Based Healthcare-Monitoring System towards Improving Quality of Life: A Review. *Healthcare*, *10*(10), 1993. https://www.mdpi.com/2227-9032/10/10/1993

Assante, D., & Fornaro, C. (2019). An Educational IoT-based Indoor Environment Monitoring System. *Proceedings of 2019 Ieee Global Engineering Education Conference (Educon)*, 1475-1479. https://doi.org/10.1109/educon.2019.8725262

Brincat, A. A., Pacifici, F., & Mazzola, F. (2019). IoT as a Service for Smart Cities and Nations. *IEEE Internet of Things Magazine*, *2*(1), 28-31. https://doi.org/10.1109/IOTM.2019.1900014 de Cerio, D. D., Hernandez, A., Valenzuela, J. L., & Valdovinos, A. (2017). Analytical and Experimental Performance Evaluation of BLE Neighbor Discovery Process Including Non-Idealities of Real Chipsets. *Sensors*, *17*(3). https://doi.org/ARTN 499

10.3390/s17030499

Dean, R. N., Craven, J. D., Guertal, E. A., & Varnavas, K. A. (2019). A PCB Sensor for Status Monitoring of Stored Food Stocks. *IEEE Sensors Letters*, *3*(4), 1-4. https://doi.org/10.1109/LSENS.2019.2902837

Dholu, M., & Ghodinde, K. A. (2018, 11-12 May 2018). Internet of Things (IoT) for Precision Agriculture Application. 2018 2nd International Conference on Trends in Electronics and Informatics (ICOEI),

Jia, M. D., Komeily, A., Wang, Y. R., & Srinivasan, R. S. (2019). Adopting Internet of Things for the development of smart buildings: A review of enabling technologies and applications. *Automation in Construction*, *101*, 111-126. https://doi.org/10.1016/j.autcon.2019.01.023

Kodali, R. K., John, J., & Boppana, L. (2020, 2-4 July 2020). IoT Monitoring System for Grain Storage. 2020 IEEE International Conference on Electronics, Computing and Communication Technologies (CONECCT),

Liu, J., Chen, C. F., Ma, Y., & Xu, Y. (2013). Energy Analysis of Device Discovery for Bluetooth Low Energy. *2013 Ieee 78th Vehicular Technology Conference (Vtc Fall)*. <Go to ISI>://WOS:000330585400159

Mishu, M. K., Rokonuzzaman, M., Pasupuleti, J., Shakeri, M., Rahman, K. S., Binzaid, S., Tiong, S. K., & Amin, N. (2021). An Adaptive TE-PV Hybrid Energy Harvesting System for Self-Powered IoT Sensor Applications. *Sensors*, *21*(8). https://doi.org/ARTN 2604 10.3390/s21082604

Shahzad, K., & Oelmann, B. (2014). A Comparative Study of In-sensor Processing vs. Raw Data Transmission using ZigBee, BLE and Wi-Fi for Data Intensive Monitoring Applications. *2014 11th International Symposium on Wireless Communications Systems (Iswcs)*, 519-524. <Go to ISI>://WOS:000363906500101

Singh, K. J., & Kapoor, D. S. (2017). A survey of IoT platforms Create Your Own Internet of Things. *Ieee Consumer Electronics Magazine*, *6*(2), 57-68. https://doi.org/10.1109/Mce.2016.2640718

Soni, N. B., & Saraswat, J. (2017). A Review of IoT devices for Traffic Management System. *Proceedings of the International Conference on Intelligent Sustainable Systems (Iciss 2017)*, 1052-1055. <Go to ISI>://WOS:000454440100182

Taskin, D., Taskin, C., & Yazar, S. (2018). Developing a Bluetooth Low Energy Sensor Node for Greenhouse in Precision Agriculture as Internet of Things Application. *Advances in Science and Technology-Research Journal*, *12*(4), 88-96. https://doi.org/10.12913/22998624/100342

Yang, J. (2019, 12-13 Jan. 2019). Design and Study of Intelligent Warehousing System Based on RFID Technology. 2019 International Conference on Intelligent Transportation, Big Data & Smart City (ICITBS),

CAPACITY OF WOMEN FARMERS IN ADOPTING THE TECHNOLOGY OF UTILIZING PREDATORY SUCKERMOUTH CATFISH AS ANIMAL FEED

Agustina Abdullah [1,2] , A.Dharmawan [1] , Aprisal [1] , Mutmainnah [1] , Sri Wira Utami, Kasmiati[3], Hastang[4], Nancy Lahay[4], Rinduwati[4] , dan Erman Asnawi [5]

[1]Vocational Faculty of Hasanuddin University, Makassar, South Sulawesi, Indonesia [1]
[2] Laboratory of Animal Husbandry Extension and Sociology, Faculty of Animal Husbandry,Hasanuddin University, Makassar,South Sulawesi, Indonesia
[3]Marine Science and Fisheries Faculty, Hasanuddin University, Makassar, Indonesia
[4]Animal Science Faculty, Hasanuddin University, Makassar, Indonesia
[5]Livestock, Animal Health and Fisheries Service Soppeng Regency, South Sulawesi Indonesia

Email: agustina.abdullah@ unhas.ac.id

ABSTRACT

The study aimed to identify the capacity of women farmers in adopting the technology of utilizing suckermouth catfish into animal feed so that it has added value through processing technology innovation and can contribute to increasing the productivity of livestock businesses. The research was conducted in Soppeng Regency, South Sulawesi Province. The determination of female farmers as respondents was done by purposive sampling, using the criteria that women have a village chicken livestock business, with a total of 30 respondents. The research data was collected by conducting a survey using collection techniques, namely interviews using questionnaires, focus group discussions, and in-depth interviews (in-depth study) with several key informants. The results showed that, in general, female farmers (80% of respondents) did not know the technology in the management of suckermouth catfish into animal feed, and more than 60% of the respondents needed the technology of processing suckermouth catfish into animal feed. However, female farmers are not well informed about the technology. It can be seen that the number of farmers who know the technology of utilizing broom- sweep fish into animal feed raw materials is still low. For this reason, efforts need to be made to improve and optimize the application of the technology of utilizing broom-sweep fish technology into animal feed raw materials in order to reduce production costs so as to increase the income and welfare of farmers in rural areas.

Keywords: : capacity of farmers (women), adoption, technology, utilization of predatory suckermouth catfish, animal feed

INTRODUCTION

The livestock sector has an important role in the rural economic system. One type of livestock that is widely cultivated in rural areas is free-range chickens. Free- range chickens

are potential germplasm and genetically have high adaptability to the environment. Iskandar S (2010) stated that free-range chicken farming is
livestock that has the potential to be developed because it can be used as a source of animal protein from eggs and meat. This indicates that free-range chickens have a fairly large role in the development of livestock in Indonesia, as well as being the economic basis for rural farmers for the meat (free-range chicken) needs of the community .

Suprayogi et al, (2018), stated that livestock business is a business that will continue. Livestock products such as meat, milk, eggs and others have extraordinary market potential. Therefore with potential Large ones require high input, especially feed requirements because the feed factor requires large input in the livestock business. Abdullah (2019) , stated that to support success in the livestock business, quality feed is an important factor. Good feed will provide good production so that optimal production can be achieved and farmers get results.

The need for quality feed is a dilemma in developing a livestock business. Therefore, a strategy is needed. application of cultivation technology and feed processing technology that utilizes local feed resources so that they can be easily absorbed by farmers in rural areas . One of the local feed resources that can be used as alternative animal feed is the utilization of predatory catfish.

Suckermouth catfish or The more familiar name Gecko fish in South Sulawesi, has not been used by the wider community because, apart from having hard skin, which makes it quite difficult to handle, the presence of the Tokke fish is also very disturbing for fishermen, because it can disrupt their activities, damage fishing gear, especially gill nets. One of the causes of this is fish develop multiply Because No own mark sell, so if caught by fishermen, they tend to be thrown away and return to the water, not utilized Because there is no interest as food fish; as a result, besides No bring income for fishermen, also resulted in disturbance health environment, so although results from the catch Lots from fishermen, this fish No own mark sell, so Lots give rise to anxiety for public mainly fisherman with the presence of broomstick fish this, though results Usman's research, 2014 stated that fresh broomstick fish that is dried and then floured has high protein content quite high up to 52.7%, even the meat protein reaches 75.5%, even though the skin is quite hard and has a fairly high ash content of around 31.9 -35.1% but Still can processed become flour. Based on matter, the so for answer problem in provision feed cattle requires effort with utilize potential resources based on potential local like the broom fish, so that the broom fish This mark plus make use of discarded broom fish that can be obtained made as material standard feed livestock that can reduce cost need livestock will feed 70 – 80% of cost production , which can pressing cost production cultivation cattle or other with more price low from feed commercial so that can increase income and welfare public breeder .

The use of broomstick fish technology as animal feed in the development of free-range chicken farming is very important, supported by a high level of technology adoption in order to increase the productivity of free-range chicken farming. However, apart from the technology for using predatory broomstick fish as animal feed, the role and capacity of breeders as farming actors, especially in adopting technology, plays an important role and the technology applied is easy to carry out/apply, the materials used are available at the breeder's location, and at a reasonable cost. cheap. Abdullah and Syamsu (2008) stated that the success of livestock business development is largely determined by the farmer's resource capacity. Development of livestock capacity is carried out by raising awareness among breeders, where all activities in livestock development are carried out by and for breeders. Farmer development is carried out with a participatory nuance so that the principles of equality, transparency, responsibility, accountability and cooperation become new contents in empowering breeders.

Empowering farmers in the livestock sector involves many women in carrying out farming businesses, especially family farming businesses. Lestariningsih et.al., (2018) stated that the role of female workers is very much needed because the livestock sector requires patience and tenacity so that female workers are more suitable. Women not only act as housewives but also as workers in the livestock sector. Many women participate or make real contributions to livestock businesses run by their families. The involvement of women in carrying out livestock activities is often carried out in free-range chicken farming businesses, because the free-range chicken farming business is an alternative business that does not require a lot of capital and large land, and can be used as a source of income for village communities in addition to a source of animal protein for the family. Abdullah and Amrawati, (2008) stated that the involvement of women in livestock farming activities is an effort to increase family economic security and efficient use of local resources.

Utilizing local resources and potential such as the predatory broomstick fish to be used as raw material for animal feed will have positive cultural, social and economic impacts, namely livestock cultivation will become more efficient with the availability of feed that can be carried out continuously and sustainably. Apart from that, the social problems that occur due to predatory fish which can become waste because they are thrown around waters which have been causing pollution can be overcome to become products that are useful as feed, and have a good economic impact on female farmers, resulting in business efficiency. Therefore, efforts are needed to develop the capacity of female breeders in utilizing existing resources, namely the use of the predatory broomstick fish so that it has added value through innovation in processing technology and can contribute to increasing the productivity of livestock businesses.

RESEARCH METHODS

The research was carried out in Soppeng Regency, South Sulawesi Province. The determination of farmer farmers as respondents was carried out using purposive sampling with a total of 30 female farmers who were engaged in raising free-range chickens as respondents. Research data was collected by conducting a survey using data collection techniques through interview questionnaires. The questions in the questionnaire are closed questions and open questions. Apart from that, focus group discussions were also held, namely conducting focus group discussions with female farmers to dig deeper and identify various actual problems and conditions that occur in the development of free-range chicken farming businesses. Apart from that, in-depth interviews were conducted with several key informants.

The variables in this research are breeder characteristics, breeder capacity (attitude, knowledge, and skills) in the free-range chicken farming business, and breeder capacity in using predatory suckermouth catfish as animal feed. Measurement of research variables is carried out by measuring indicators for each research variable/sub-variable in question. Qualitative variable indicators are measured using a Likert scale consisting of four levels, each given a score of 1, 2, 3 and 4. The measurement of each indicator is obtained by taking the average value of the scores of all parameters. Analysis of existing condition data obtained from the survey begins by tabulating the data, and carrying out descriptive analysis of the data by looking at means, percentages and frequencies which are processed with the help of SPSS software.

RESULTS AND DISCUSSION

The general characteristics of respondent farmers are related to age, education, family size, farming experience, and livestock ownership as shown in Table 1. Table 1 shows that most of the breeders are in the range of 31-50 years with 70% of all respondents. This shows that female farmer respondents who work in developing free-range chicken farming businesses are in the productive age category. The age factor is usually identified more with work productivity, and if someone is still of productive age, their productivity tends to be high. Age is an indicator that shows a person's physical abilities. The age of the workforce influences work productivity, age is also closely related to the mindset of workers in determining the management system that will be applied in activities or at work. Abdullah and Amrawati, (2008) stated that the younger the breeder (productive age 20-45 years), generally the curiosity about something is higher and the interest in adopting the introduction of technology is higher.
The level of education has an influence on livestock businesses both technically, management and management of livestock businesses in absorbing new technology. With a high level of education it is hoped that breeders will be able to carry out their livestock business activities better because they are supported by increasingly broader knowledge and insight (Lestariningsih, et al. 2018) . Judging from the level of formal education, there are variations from the lowest not having completed elementary school and the highest having completed college. The education level of breeders is dominated by those who have not completed or completed elementary school (36.7), the remainder have completed junior and senior high school respectively 33.3 % and 26.7 %, and only 3.3 % have completed their education at university. The higher the farmer's education level, the higher the quality of human resources, which in turn will be the higher the productivity of the work they do . With this level of education, it can be assumed that the farmer's ability to know and adopt skills in the context of developing a livestock business will experience obstacles and difficulties. An adequate level of education will have an impact on increasing the performance and management capabilities of the livestock business being run (Zahidah, et al., 2021). The level of education will increase knowledge and skills so that it will increase work productivity and will determine the success of a livestock business so education is very much needed in running a business because it has an impact on the mindset in managing a chicken farming business (Sudrajat and Isyanto, 2018).
Based on the level of experience of farmers, the research results show that the majority of female farmers have experience in raising chickens and ducks for up to 10 years (60.%) of the total respondents , and the remaining 40.0 % have experience in raising more than 10 years . Generally, experience in raising free- range chickens is passed down from their parents from generation to generation.

Table 1. The general condition of respondent farmers

No	Description	Number of people)	Percentage (%)

1	Breeder Age (years)		
	< 30	6	20.0
	31 - 40	10	33.3
	41 - 50	11	36.7
	>50	3	10.0
2	Education		
	Did not graduate/finished elementary school	11	36.7
	Finished high school	10	33.3
	Finished high school	8	26.7
	Graduated from College	1	3.3
3	Number of Families (people)		
	< 3	5	16.7
	3 – 4	8	26.7
	5 – 6	10	33.3
	> 6	7	23.3
4	Ownership of livestock (tail)		
	<10	5	16.7
	10-15	9	30.0
	16-20	10	33.3
	>20	6	20.0
5	Livestock experience (years)		
	≤5	8	26.7
	>5-10	10	33.3
	>10-15	9	30.0
	>15	3	10.0

Having long-term farming experience provides an indication of the knowledge and skills of female farmers regarding the management of free-range chicken farming have better abilities. This is in accordance with the opinion of Kurniawati, et al., 2022) , that farming experience is an important capital for the success of a farm, but the level of experience of each farmer is different as well as their mindset in applying innovation to their livestock business activities .
Characteristics of the ability or knowledge capacity and skills of female farmers in the free-range chicken farming business are as shown in Figure 1.

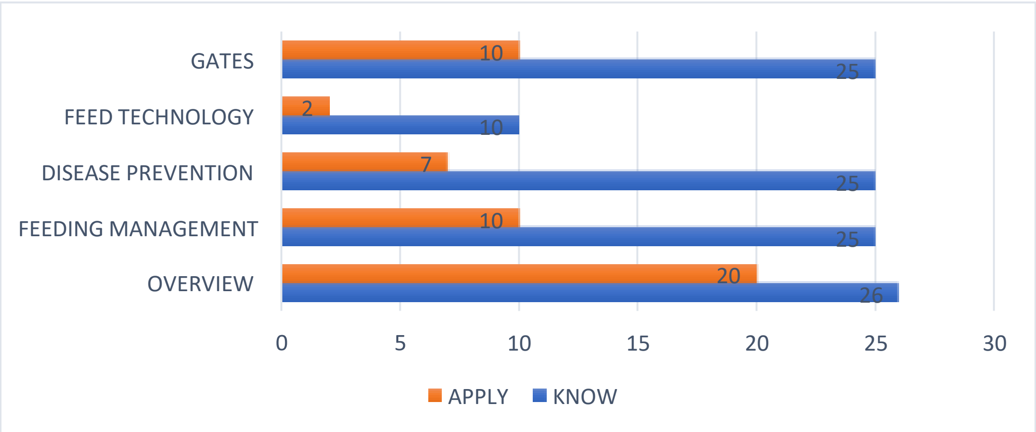


Figure 1. Knowledge capacity of farmers in managing chicken farming businesses village

Figure 1 shows that female farmer respondents know quite high cage technology, 83%, but the % of farmers who apply it is 30.2% of those who know. This shows that farmer knowledge of cages is quite good, but implementing cage management is still lacking; this is because the business of raising native chickens in rural areas is more extensive, so farmers only let their livestock out by utilizing the yard as a livestock pen. Likewise, farmer knowledge in preventing disease is still lacking; respondents are of the opinion that native chickens are rarely affected by disease. This is in accordance with (Setianan, 2010), which states that native chickens have characteristics that are easy to maintain and rarely get sick, so it is one of the advantages of native chickens. In addition, native chickens have characteristics that are more popular with certain market segments. For farmer knowledge of feed technology and feed management, farmers have known about the technology more than 50%, but the number of farmers who apply it is still less than 50% of those who know. This shows that in general, the respondent farmers have a good understanding of the technology in managing native chicken farming businesses such as cages, feed technology, disease prevention, feed management and hatching as much as 80%, but they are still lacking in the application of the technology they know.
Figure 2 shows the characteristics of farmers' capacity in terms of knowledge and skills in utilizing local broomstick fish resources as raw material for free-range chicken feed. In general, it appears that more than 80% of the respondents do not know that broomstick fish can be used as raw material for animal feed. Thus, breeders do not know well about this technology. This shows that the number of breeders who know the technology is still less than 20%, and only 10% of those who know about the use of broomstick fish technology as a raw material for animal feed apply it. For this reason, efforts need to be made to improve and optimize the application of free-range chicken farming technology by using broomstick fish as a source of raw material for free-range chicken livestock feed at the community farm level.

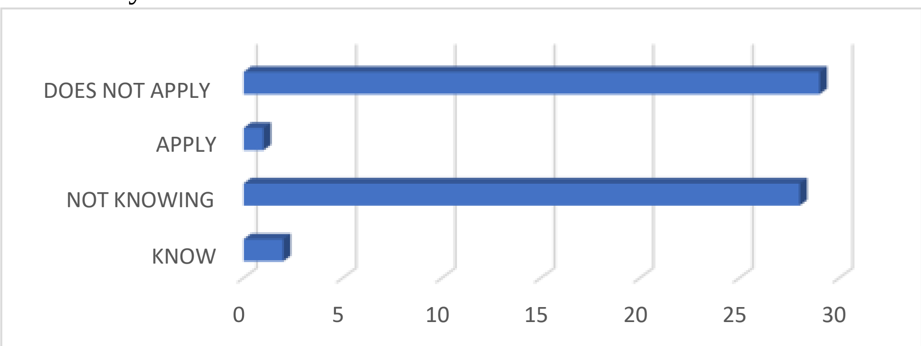


Figure 2. Characteristics of technological knowledge capacity for using broomstick fish as raw material for free-range chicken feed

CONCLUSIONS AND RECOMMENDATIONS

In general, female farmers (80% of respondents) already know the cultivation technology of native chicken businesses but are still lacking in applying the technology. The same applies to the technology of utilizing suckermouth catfish as raw material for animal feed. More than 60% of the respondents need technology to utilize predatory fish as raw material for animal feed. Thus, it turns out that farmers are not well informed about the technology. This can be seen from the low number of farmers who know the technology, which is 20%. For this reason, efforts need to be made to improve and optimize the application of village chicken business technology, as well as the utilization of predatory broom fish as raw material for animal feed products at the smallholder farm level.

ACKNOWLEDGMENT

The authors would like to thank the Director General of Vocational and the Rector of Hasanuddin University for the research funding assistance through the Fiscal Year 2024 Matching Fund Program, so that this research can be carried out.

REFERENCES

Abdullah, A. and Amrawati, AA 2008. The role of free-range chicken farmers' wives in an effort to increase family income in Bulukumba Regency. Proceedings of the National Seminar on Animal Husbandry and Veterinary Technology. Faculty of Animal Husbandry. Hasanuddin University. Makassar. 711-717.

Abdullah, A and Syamsu, JA 2008. Strengthening Livestock Farmer Groups in the Development of Livestock Agribusiness. Bulletin Farm . Issue 28 Animal Husbandry Province . Suawesi - South , Makassar

Abdullah (2019). Adoption of beef cattle feed technology from a scientific perspective Counseling, Unhas Press, 2019 Iskandar, S. 2010. Village Chicken Farming Business. Editor: Ketaren, PP, Sopiyana. S., Sudarman.

D. Bogor: Ciawi livestock research center.

Kurniawati, AD, Mukson, and SI Santoso. 2022. Analysis of Income and Factors that Influence the Laying Chicken Farming Business in Klaten Regency. Agroland: Journal of Agricultural Sciences. 29(3):219-229.

Lestariningsih, M., Bazuki., Y. Endang. 2018. The role of women dairy farmers in improving the family's standard of living. Equity Journal. 12(1): 121-41.

Setianan .2010.Study of the role of free-range chicken farmers in rural areas in supporting food security and sovereignty. Proceedings of the National Seminar on Local Poultry IV.55-88

Sudrajat, and AY Isyanto. 2018. Factors that Influence the Income of Sentul Chicken Farming Businesses in Ciamis Regency. Journal of Scientific Community Thought with an Agribusiness Insight. 4(1): 70-83.

Zahidah, K., M. Sugiarto, and YN Wakhidati. 2021. Factors Associated with the Independence of Partner Farmers in the Broiler Chicken Business in Banyumas Regency. Journal of Animal Science and Technology. 3(3):323-331.

Umar , H. 1997. Research Methodology : Application in Marketing. PT Gramedia, Jakarta

CONGENITAL LUXATION OF THE HIPS AND SHOULDERS IN A TOY BREED DOG: CASE REPORT

Taulant GOGA[1,2], Erinda LIKA[1], Barbara CARULLI[2], Bledar GOXHA[1,2], Alberto CROVACE[3]

1. *Faculty of Veterinary Medicine, Agricultural University of Tirana, Tirana, Albania.*
2. *Department of Precision and Regenerative Medicine and Ionian Area, University of Bari "Aldo Moro", Bari, Italy.*
3. *Department of Veterinary Medicine, University of Sassari, Sassari, Italy*

Corresponding author e-mail: taulantgoga@gmail.com

ABSTRACT

This case report presents a rare condition in dogs involving congenital bilateral shoulder luxation and bilateral hip luxation, primarily affecting toy breeds. It is characterized by a malformed glenoid cavity, congenital dislocation of hips, leading to persistent discomfort and lameness. Hip dislocation, usually craniodorsal, is more common, typically resulting from trauma or hip dysplasia. Observing both congenital bilateral shoulder and hip dislocations at the same time is rare. This case report refers to a 4-year-old male, toy breed dog presented with severe right shoulder pain and reduced range of motion, moderate back limbs lameness. Radiographic examinations shows bilateral congenital luxation in the shoulder joints with ipoplastic glenoid cavity and humeral head deformity and congenital luxation of both hip joints with bilateral flattening of the acetabular cavities and severe modification of femoral heads. The dog was diagnosed with bilateral hip and shoulder congenital luxation. The right shoulder showing more instability and severe pain and lame. There is no definitive evidence directly linking hip and shoulder luxation, genetic predispositions and environmental factors contributing to overall joint health can influence the occurrence of both conditions in dogs. Surgical intervention was required to correct the dislocations by using the Vaughan technique for extracapsular stabilization of the shoulder, Femoral Head Excision (FHO) technique was performed for the stabilization of hip luxation, followed by a strict rehabilitation plan. This report discusses the clinical presentation, diagnostic process to evaluate this condition, surgical approaches, and post-operative outcomes, emphasizing the challenges posed by managing such cases in small toy breeds. The simultaneous congenital dislocation of the hip and shoulder joints in this case is important since it is a case of a condition not before reported in the literature, highlighting the importance of an adequate orthopedic assessment in toy breeds.

Keywords: Congenital, Hip luxation, Shoulders luxation, Extracapsular stabilization.

INTRODUCTION

In dogs, congenital shoulder luxation is an uncommon disorder that frequently affects both shoulders. It is characterized by a malformed or hypoplastic glenoid cavity, persistent discomfort, and lameness. Small and toy breed dogs are prone to medial congenital luxations(Ciro Marra et al., 2018). Hip dislocation is the most frequent type of dislocation in dogs and is generally caused by a traumatic event or secondary to hip dysplasia(Demko et al., 2006; Kieves et al., 2014). The majority parts of cases are due to road traffic accidents or secondary to hip dysplasia. The direction of luxation is mostly craniodorsal due to the strength of gluteal muscles(Basher et al., 1986; Bone et al., 1984).
Rarely, dislocation can be congenital and due to its rarity, it is poorly reported in the literature and is associated with missing of acetabular cavity and serious osteoarticular changes. Observing a

congenital bilateral shoulder dislocation and a bilateral hip dislocation at the same time is an event that should be considered very rare.

Clinical Case Description:

A male dog, 4 years old, Barebone Toy breed was presented to the hospital of Surgery and Animal Reproduction Section, University of Bari "Aldo Moro" with the anamneses below: As a puppy he showed a slight lameness in the back limbs but that time the owners thought that it was not a serious situation regarding to the young age and growing time of the dog. At the age two, he began to complain sporadically of pain while was playing, walking and running. The dog was brought for clinical visit due to severe pain in the right shoulder on which a serious functional disorder was observed with reduced range of motion and difficulty in extension. Orthopedic evaluations of the contralateral limb demonstrated joint instability but there was no severe pain so the animal transfer weight on this limb. After the short anamneses indicated by the owner and the orthopedic visit, we decided to perform X-rays to the anterior and posterior limbs, to evaluate first of all the pain of the posterior limbs, even the pain in neck and difficulty in extension of anterior limbs. From this radiographic examination of the pelvis and shoulders, we have some incongruency evidences in the articular joints between head of humerus and glenoidal cavity of shoulders (Figure 1.B and 1.C) and also both head of femurs were out of acetabulum (Figure 1.A).

Figure 12 Radiographic examination: A) VD projection of hip joint, B) DV projection of left shoulder joint, C) DV projection of right shoulder joint

After the radiographic examination and interpretation of X-rays findings, the dog was diagnosed with bilateral hip luxation and bilateral shoulders luxation. Regarding bilateral hip luxation, the right head of femur was more craniodorsal and out of acetabulum than the left one. In front limbs were presence of bilateral shoulders luxation and flattening of glenoid cavity, the head of humerus were deformed and out of glenoid cavities. The right limb showed severe pain and lame and for that reason we decided to stabilize the shoulders. The surgical technique used for treatment of shoulders luxation was Vaughan technique(Vaughan, 1967) as showed in Figure 2, that consist in an extracapsular stabilization used also for the stabilization of the recurrent dislocation of the shoulder joint . About the stabilization of back limbs was chosen to perform the FHO (Femoral Head Ostectomy) technique which consist in the excision of femoral head of both back limbs, after that the gluteal muscles around will create a false joint around this articulation and the animal will be pain free.
All the surgeries were not performed in the same time but step by step starting from shoulders, one surgery every 1 or 2 months after the previous surgery depending from the condition of dog, so in this way the dog can hold the body weight in three limbs.

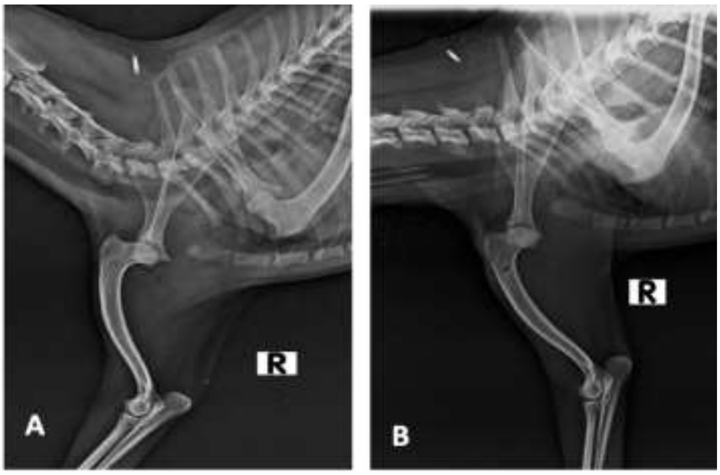


Figure 2 Right Shoulder articulation: A - Before surgery, B - After surgery

DISCUSSION AND CONCLUSION

This clinical case was selected like a case report because is a rare case in which is presented in the same time an orthopedic pathology known as congenital luxation of hip and congenital luxation of shoulders joints, also because is a congenital pathology in toy breeds due to the presence of strange structure of glenoidal cavity. There is no definitive evidence directly linking hip and shoulder luxation, genetic predispositions and environmental factors contributing to overall joint health can influence the occurrence of both conditions in dogs. There are some correlations between others pathologies as osteoarthritis and dysplasia in articulation structure. According to the study of *Swenson et al.,1997, Maki et al., 2000, 2002* two prevalent developmental orthopedic abnormalities in dogs that can result in permanent impairment are elbow and hip dysplasia. Both conditions are regarded as complicated illnesses that are influenced by a combination of environmental variables and several genes(Mäki1 et al., 2002; Swenson et al., 1997). Also, a case report from *Castelli et al., 2019*, show a correlation of hip luxation with patellar luxation in a toy breed dog(Castelli et al., 2019). So, in this case there are no bibliographic evidences regarding to the presence of bilateral congenital shoulder luxation and bilateral congenital hip luxation in the same time. In our daily work we have been confronted with some congenital and non-congenital diseases in toy breed dogs that have been presented even before and starting from this case report we have to be more careful during the orthopedic evaluation and to see if there is any correlation between different structures. This case report serves as an initial signal of a scientific research regarding bone pathologies, mainly articulations and correlations that may exist in the presence of the same pathology in different bone structures.

REFERENCES

Basher, A. W. P., Walter, M. C., & Newton, C. D. (1986). Coxofemoral Luxation in the Dog and Cat. *Veterinary Surgery, 15*(5), 356–362. https://doi.org/10.1111/j.1532-950X.1986.tb00243.x

Bone, D. L., Walker, M., & Cantwell, H. D. (1984). Traumatic Coxofemoral Luxation In Dogs Results of Repair. *Veterinary Surgery, 13*(4), 263–270. https://doi.org/10.1111/j.1532-950X.1984.tb00807.x

Castelli, E., Longo, F., & Pozzi, A. (2019). Treatment of a hip and patella luxation in a toy dog. *Schweiz Arch Tierheilkd, 161*(12), 831–836. https://doi.org/10.17236/sat00239

Ciro Marra, Cappellari, F., & Panichi, E. (2018). *Treatment of congenital shoulder luxation associated with swimmer syndrome in a puppy*. *32*(2), 1–5.

Demko, J. L., Sidaway, B. K., Thieman, K. M., Fox, D. B., Boyle, C. R., & McLaughlin, R. M. (2006). Toggle rod stabilization for treatment of hip joint luxation in dogs: 62 cases (2000–2005). *Journal of the American Veterinary Medical Association*, *229*(6), 984–989. https://doi.org/10.2460/javma.229.6.984

Kieves, N. R., Lotsikas, P. J., Schulz, K. S., & Canapp, S. O. (2014). Hip Toggle Stabilization Using the TightRope® System in 17 Dogs: Technique and Long-Term Outcome. *Veterinary Surgery*, *43*(5), 515–522. https://doi.org/10.1111/j.1532-950X.2014.12158.x

Mäki1, K., Groen, A. F., Liinamo, A.-E., & Ojala, M. (2002). Genetic variances, trends and mode of inheritance for hip and elbow dysplasia in Finnish dog populations. *Animal Science*, *75*(2), 197–207. https://doi.org/10.1017/S1357729800052966

Swenson, L., Audell, L., & Hedhammar, Å. (1997). Prevalence and inheritance of and selection for elbow arthrosis in Bernese Mountain Dogs and Rottweilers in Sweden and benefit:cost analysis of a screening and control program. *Journal of the American Veterinary Medical Association*, *210*(2), 215–221. https://doi.org/10.2460/javma.1997.210.02.215

Vaughan, L. G. (1967). Dislocation of the Shoulder Joint in the Dog and Cat. *Journal of Small Animal Practice*, *8*(1), 45–48. https://doi.org/10.1111/j.1748-5827.1967.tb04497.x

EVALUATION OF POLYPHENOLS AND ANTHOCYANINS CONTENT IN KALLMET WINE, THROUGH SEQUENTIAL FERMENTATION, USING SELECTED NON-SACCHAROMYCES YEAST

Mamica Ruci[1], Renata Kongoli[2], Massimo Iorizzo[3], Bruno Testa[3], Julian Karaulli[1], Fatbardha Lamçe[1], Rozarta Nezaj[1], Onejda Kyçyk[1]

[1] Faculty of Biotechnology and Food, Food Research Center, Agricultural University of Tirana, Albania
[2] Faculty of Biotechnology and Food, Department of Agro-food Technology, Agricultural University of Tirana, Albania
[3] Department of Agriculture, Environmental and Food Sciences, University of Molise, 86100 Campobasso, Italy

Corresponding author e-mail: mruci@ubt.edu.al

ABSTRACT

This study investigates the effects of sequential fermentation using selected non-Saccharomyces (*M. pulcherrima*) and Saccharomyces yeasts on the polyphenols and anthocyanin content in red wines. The consumers interest in quality wines with enhanced health benefits and richer color profiles is growing, therefore understanding the role of different yeast strains in wine fermentation and modification of vinification schemes become increasingly important. This research involves conducting fermentation trials where *M. pulcherrima* selected strain are initially used at a concentration of $log10^7$, followed by commercial strain of Saccharomyces cerevisiae in a sequential manner added after 48 and 72 hours. A control trial used only the Saccharomyces cerevisiae strain. The total polyphenols and anthocyanin content of resulting wines are analyzed using the spectrophotometry. The results demonstrated that sequential fermentation significantly boosts these compounds compared to traditional fermentation methods, leading to wines with improved antioxidant properties and deeper color intensity. These first findings suggest that the utilization of selected non-Saccharomyces yeasts in conjunction with Saccharomyces, can enhance the quality and health benefits of red wines, offering valuable insights for winemakers seeking to innovate and improve their products.

Keywords: polyphenol, anthocyanin, color intensity, non-saccharomyces yeasts.

INTRODUCTION

Albania has a rich history of viticulture, dating back to ancient times. The country's Mediterranean climate, characterized by warm summers and mild winters, along with diverse soil types, makes it ideal for growing a variety of grapevines. Among the indigenous varieties, Kallmet stands out as one of the most important red grapes in Albania. Mostly cultivated in northern areas, especially in the districts of Lezhë, Shkodër, and Mirdita. Nowadays, the consumer's interest in quality wines with enhanced health benefits and richer color profiles is growing, therefore understanding the role of different yeast strains in wine fermentation and modification of vinification schemes become increasingly important. Several factors impact wine quality, including grape variety, vineyard management, climatic conditions, soil composition, winemaking procedures, and yeast strain selection (Pangzhen Zhang 2021). These components work together to impact the wine's fragrance, taste, color, and texture, establishing its overall character and aging potential. Kallmet wines are usually made with commercial Saccharomyces cerevisiae yeast, producing high-quality dry wines with high alcohol concentration (13-15%). Previous research has shown that Kallmet wines contain a sufficient amount of anthocyanin and a reasonably high mean degree of polymerization (mDP). The wine's color is largely attributed to polymerized pigments, which ensure good color stability during storage (Peçuli 2018). However, there is a lack of studies in Albania on the impact of innovative winemaking techniques, particularly sequential fermentation with selected non-Saccharomyces yeasts, on the polyphenol content and color stabilization in Kallmet red wine. Maceration, sulfiting, temperature, and microbial populations all have an impact on the amount and content of polyphenols in red wines (Ana C. Correia 2018). Hornedo-Ortega, 2021). Polyphenols are a class of naturally bioactive substances found in grapes that influence the flavor, color, and mouthfeel of wine. They are split into two categories: flavonoids and nonflavonoids (D. Violeta Ivanova, 2011). Polyphenols contain antioxidant capacities that are recognized for their potential positive health effects. Anthocyanins are a kind of polyphenol responsible for the color red, purple, and blue in grapes. They play a crucial role in the color and stability of red wines (Raúl Ferrer-Gallego 2014). Recent research has highlighted the importance of selecting yeast strains not only for their fermentation capabilities but also for their ability to enhance the wine’s color, texture, aroma, and potential health benefits (Antonio Morata 2016, Anna L. Carew 2013, Pangzhen Zhang 2021). In modern winemaking, certain strains of Saccharomyces cerevisiae and non-Saccharomyces yeasts are often used to accomplish desired results. These yeasts influence wine's final color characteristics through a variety of mechanisms, including the release of pectinase and beta-glycosidase during maceration, which can affect phenolic extraction from grapes and hydrolyze the glycosidic bonds of phenolic compounds, respectively (Alimardani-Theuil 2011). Furthermore, yeasts can promote chemical interactions between their cell walls and polyphenols. Previous study has shown that various non-Saccharomyces yeasts used in sequential inoculation with Saccharomyces cerevisiae can generate different aroma profiles and increase the phenolic content of red wines. (Morata Antonio 2016; R. Escribano 2018).

The purpose of this study is to explore into the impact of sequential fermentation with selected non-Saccharomyces (M. pulcherrima) and Saccharomyces yeasts on the polyphenol and anthocyanin content of Kallmet wine. This study will involve fermentation trials in which M. pulcherrima selected strains are utilized at a log107 concentration, followed by commercial Saccharomyces cerevisiae strains introduced sequentially after 48 and 72 hours. A control test only utilized the Saccharomyces cerevisiae strain. The total polyphenol and anthocyanin content of the resultant wines are measured using spectrophotometry techniques.

MATERIALS AND METHODS

Yeast Strains and Fermentation trails

In this study is used *M. pulcherrima* (AS3C1) strain isolated from grape must and belongs to the culture collection of the Di.A.A.A (Department of Agricultural, Environmental and Food Sciences, University of Molise, Campobasso, Italy). Before use the strain it was grow in YPD medium at 20^0C under aerobic condition for 48 h. The commercial strain of *S. cerevisiae Zymaflore F15 (*LAFFORT Co., Bordeaux, France) was used as control yeast. The cultures were centrifuged at 5000 rmp for 10 min at 4 ^{0}C, washed twice with sterile physiological solution (0.9% NaCl) before used, and inoculated in Kallmet grape juice to concentration of 7.0 log CFU/ml. For the fermentation trails red grapes (*Vitis vinifera* cv. Kallmet) were taken in North of Albania, at Koplik village in collaboration with “MEDAUR” winery. The fermentation trails are conducted at Research Food Center, faculty of Biotechnology and Food. The grape juice was used for three different schemas of inoculation, first trail was initially inoculated with *M. pulcherrima* (10^7 CFU/ml) and after 48 h was added the S. *cerevisiae* F15 to finish the fermentation. In second trail the S. *cerevisiae* F15 was added after 72 h and the third trail was fermented only with S. *cerevisiae* F15 as control test. The alcoholic fermentation process was conducted at control temperature 23-24ºC and was monitored every day by measuring the sugar consumption and temperature.

Chemical analyses and Polyphenols

At the end of alcoholic fermentation the wine from every trails was subject to chemical analysis pH, alcohol (%v/v), total acidity (g/L ac. Tartaric), and volatile acidity (mg/L ac. Acetic) were performed according to International Organization of Vin and Wine (OIV) methods. Glycerol and acetaldehyde were determined using enzymatic kit according the instruction of the company. Total polyphenols were analyzed with spectrophotometer according to the method (M. S. Violeta Ivanova 2010) with some modification using The Folin–Ciocalteu. The absorbance of the samples was measured at a fixed wavelength of 750 nm against distilled water as the blank. A calibration curve was constructed using Gallic acid standard solutions (0–100 mg/L). The concentration of total phenolic is expressed as the Gallic acid equivalent ml/ L. All samples were prepared in triplicate. The total anthocyanin content was analyzed according to Puissant-Leon (Puissant and Léon, 1967). The samples were diluted with a solution of 1N HCL and the absorbance was measured at 520nm. The color intensity and tonality was determined by measurement the absorbance of wine samples directly at 420, 520 and 620 nm using a 2 mm optical path according (M. S. Violeta Ivanova 2010). From this was calculated the color intensity (CI) as sum of the absorbance at 420, 520 and 620 nm, tonality or tint (CT) was calculated as the ratio A420/A520, and gives a measure of the "tint" or redness of the wine.

Statistical Analysis

Analysis of variance (ANOVA) was applied for the data obtained from the experiments, using the SPSS software. The data represent three replicates for each trial fermentation (n=3). The data were considered significant if P-values ≤ 0.05.

RESULTS AND DISCUSSION

Chemical parameters and Polyphenols

The result of chemical parameters and polyphenols content for the samples of the wines obtain from the alcoholic fermentations from three trails are presented in able 1. The data obtained indicate that **MP48h** (sequential fermentation with M pulcherrima) exhibited significantly higher glycerol content and lower levels of volatile acidity and acetaldehyde compared to the second trail **MP72h** and control trial **SC3**. These results are consistent with previous studies on the use of MP (*Metschnikowia pulcherrima*) in sequential fermentation, highlighting its beneficial role in improving the chemical composition of wine (Chen 2018, Testa 2021).

Polyphenols are important compounds in wine that contribute to its organoleptic and antioxidant properties. The result among the samples, MP48h has the highest concentration of total polyphenols, compare to MP72h and control test SC3. Anthocyanins are pigments that contribute to the red, purple, and blue hues in wine. Test MP48h and MP72h has the highest anthocyanin content, indicating it might have the most intense red color and potentially better color stability compare to control SC3 which has the lowest anthocyanin content, suggesting it may have a lighter color compared to the others

Table 1: Chemical parameters and Polyphenol content obtain from sequential fermented of Kallmet red wine: SC3 (control fermentation); MP48h (sequential fermentation M.pulcherrima + S.cereviseae); MP72h (sequential fermentation M.pulcherrima + S.cereviseae)

Parameters	SC3	MP48h	MP72h
pH	3.69±0.005	3.75±0.026	3.72±0.005
Total acidity (g/L ac. Tartaric)	5.2±0.100	5.6±0.057	5.8±0.100
Volatile acidity(g/L ac acetic)	0.40±0.015	0.24±0.010	0.29±0,010
Alcohol (% v/v)	12.34±0.05	12.0±0.11	11.79±0.010
Glycerol (mg/L)	3.0±0.057	4.5±0.057	3.5±0.057
Acetaldehyde (mg/L)	10.22±0.011	4.23±0.057	6.27±0.010

Total Polyphenols (mg/L)	2520±0.577	2640±0.577	2520±0.577
Anthocyanin (mg/L)	199.03±0.01	261.17±0.025	227.03±0.060
Colour intensity (CI)	5.97±0.057	9.98±0.057	10±0.057
Tonality (T)	0.66±0.000	0.77±0.000	0.8±0.000

Data are express as mean values (n=3) ±standard deviation
.

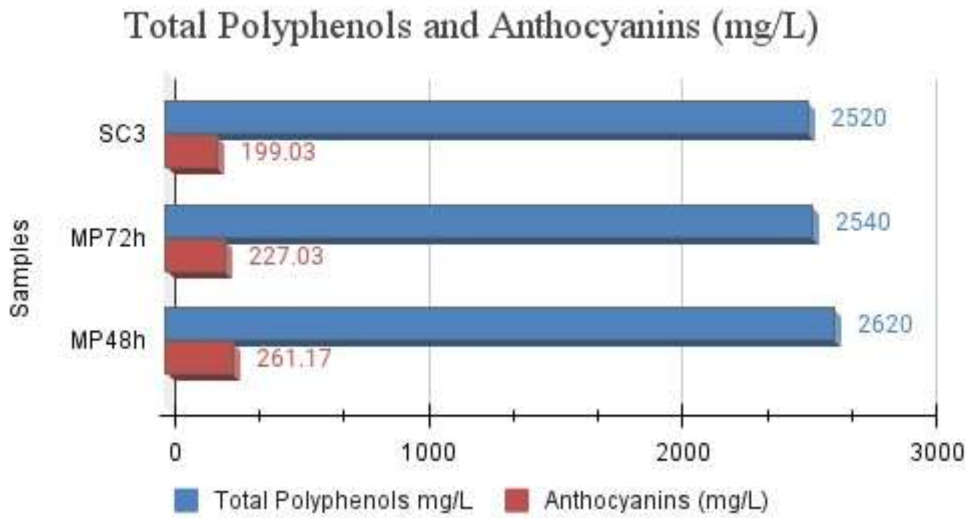


Figure 1 Total Polyphenol and Anthocyanin content on the samples of Kallmet wines

Color intensity is a measure of how dark or intense the wine's color is. Tonality provides information about the wine's hue. From the results MP72h and MP48h have similar and higher tonality and color intensities, indicating they are likely darker wines, while SC3, with a lower CI of 5.97 and tonality, is probably lighter in color.The results for color intensity (CI) and Tonality (T) for the analyzed sample are presented in Figure1.

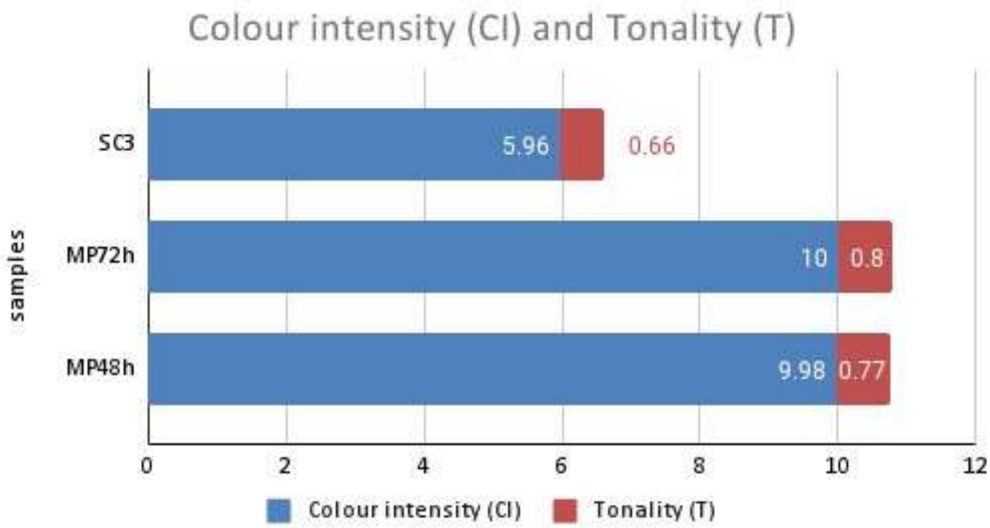


Figure 2 Color Intensity and Tonality on the samples of Kallmet wines

The use of M. pulcherrima starter in combination with S.cerevisiae can contributed to improve the color and phenolic content in wine due to its reach enzymatic activity such has been reported in previous researcher (Anna L. Carew 2013, Antonio Morata 2016, Ana C. Correia 2018).

CONCLUSION

Sequential fermentation with selected non-*Saccharomyces* yeast strains represents a promising green strategy for addressing challenges in winemaking, such as enhancing flavor complexity, improving wine stability, and reducing the need for chemical additives, while promoting more sustainable production practices.

This is the first research in Albanian winemaking to use a particular strain of Metschnikowia pulcherrima in the sequential fermentation of Kallmet wine, with the aim of analyzing its effects on chemical parameters and color composition. The findings suggest that this approach can result in high-quality wine with a fuller body, color, and aromatic complexity.

Future study will look at the volatile and non-volatile compounds found in these wines using advanced methods like Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC). Furthermore, will conducted sensory evaluations by an expert panel to completely analyze the wines' sensory characteristics and overall quality.

REFERENCES

A.B. Bautista-Ortín, J.I. Fernández-Fernández, J.M. López-Roca, E. Gómez-Plaza,. "The effects of enological practices in anthocyanins, phenolic compounds and wine colour and their dependence on grape characteristics." *Journal of Food Composition and Analysis*, 2007: 546-552.

Ana C. Correia, Fernanda Cosme, António M. Jordão. "Impact of Modern Winemaking Technologies on Phenolic Composition of Red Wines." In *A Closer Look at Grapes, Wines and Winemaking*, by Joni D. Perez. New York: Nova Science Publisher , 2018.

Anna L. Carew, Paul Smith, Dugald C. Close, Chris Curtin, and Robert G. Dambergs. "Yeast Effects on Pinot noir Wine Phenolics, Color, and Tannin Composition." *Journal of Agricultural and Food Chemistry*, 2013.

Antonio Morata, Iris Loira, José María Heras, María Jesús Callejo, Wendu Tesfaye, Carmen González, José Antonio Suárez-Lepe. "Yeast influence on the formation of stable pigments in red winemaking." *Food Chemistry,* 2016: 686-691.

Bakker, J., Bridle, P., Bellworthy, S. J., Garcia-Viguera, C., Reader, H. P., & Watkins, S. J. "Effect of sulphur dioxide and must extraction on colour, phenolic composition and sensory quality of red table wine." *Journal of the Science of Food and Agriculture,* , 1998: 297-307.

Chen, K., Escott, C., Loira, I., Del Fresno, J. M., Morata, A., Tesfaye, W., ... & Benito, S. "Use of non-Saccharomyces yeasts and oenological tannin in red winemaking: Influence on colour, aroma and sensorial properties of young wines." *Food Microbiology,* 2018: 51-63.

Hornedo-Ortega, R., Reyes González-Centeno, M., Chira, K., Jourdes, M., & Teissedre, P.-L. *Phenolic Compounds of Grapes and Wines: Key Compounds and Implications in Sensory Perception.* IntechOpen. doi: 10.5772/intechopen.93127, 2021.

J.A. Suárez-Lepe, A. Morata,. "New trends in yeast selection for winemaking." *Trends in Food Science & Technology*, 2012: 39-50.

Morata Antonio, Loira Iris, Suárez Lepe Jose Antonio. *Influence of Yeasts in Wine Colour.* Grape and Wine Biotechnology, 2016.

Pangzhen Zhang, Wen Ma, Yiqi Meng, Yifan Zhang, Gang Jin, Zhongxiang Fang. "Wine phenolic profile altered by yeast: Mechanisms and influences." *Comprehensive Reviews in Food Science and Food Safety* , 2021.

Parissa Alimardani-Theuil, Angélique Gainvors-Claisse, Francis Duchiron. "Yeasts: An attractive source of pectinases—From gene expression to potential applications: A review." *Process Biochemistry,* 2011: 1525-1537.

Peçuli, A., Lorenzini, F., Angjellari, M., Schnee, S., Gindro, K., & Dienes-Nagy, Ágnes. "Polyphenolic profile and stilbene content of Albanian “Kallmet” monovarietal wine. ." *OENO One,*, 2018: 135–144.

R. Escribano, L. González-Arenzana, J. Portu, P. Garijo, I. López-Alfaro, R. López, P. Santamaría, A.R. Gutiérrez. "Wine aromatic compound production and fermentative behaviour within different non-Saccharomyces species and clones." *Journal of Applied Microbiology*, 2018: 1521–1531.

Raúl Ferrer-Gallego, José Miguel Hernández-Hierro, Julián C. Rivas-Gonzalo, M. Teresa Escribano-Bailón. "Sensory evaluation of bitterness and astringency sub-qualities of wine phenolic compounds: synergistic effect and modulation by aromas." *Food Research International,* 2014: 1100-1107.

Testa, Bruno, Francesca Coppola, Silvia Jane Lombardi, Massimo Iorizzo, Francesco Letizia, Massimo Di Renzo, Mariantonietta Succi, and Patrizio Tremonte. " "Influence of Hanseniasporauvarum AS27 on Chemical and Sensorial Characteristics of Aglianico Wine." *Processes* , 2021.

Violeta Ivanova, Ágnes Dörnyei, László Márk, Borimir Vojnoski, Trajće Stafilov, Marina Stefova, Ferenc Kilár. "Polyphenolic content of Vranec wines produced by different vinification conditions." *Food Chemistry*, 2011: 316-325.

Violeta Ivanova, Marina Stefova, Fabio Chinnici. "Determination of the polyphenol contents in Macedonian grapes and wines by standardized spectrophotometric methods." *J. Serb. Chem. Soc*, 2010.

Violeta Ivanova-Petropulos, Arianna Ricci, Dusko Nedelkovski, Violeta Dimovska, Giuseppina P. Parpinello, Andrea Versari,. "Targeted analysis of bioactive phenolic compounds and antioxidant activity of Macedonian red wines." *food chemistry*, 2015: 412-420.

MEETING THE ENERGY NEEDS IN LIVESTOCK ENTERPRISES WITH HYBRID RENEWABLE ENERGY SYSTEMS AND ITS ENVIRONMENTAL EFFECTS

Elif Türkboyları[1], Ahmet Nedim Yüksel[2]*

[1]*Vocational School of Technical Sciences, Department of Plant and Animal Production, Tekirdağ Namık Kemal University, Tekirdağ, Turkey*
[2]*Faculty of Agriculture, Department of Biosystem Engineering, Tekirdağ Namık Kemal University, Tekirdağ, Turkey (Emeritus)*

**Corresponding author's e-mail: eyuksel@nku.edu.tr*

ABSTRACT
The most important way to combat global climate change is to use renewable energy sources as much as possible instead of traditional energy. Using renewable energy sources instead of fossil energy makes a significant contribution to countries' energy policies in every field. Due to our country's location in the world, it is in a very good situation in terms of solar energy and wind energy in coastal areas. It is suitable for generating electrical energy with wind turbines and solar panels. In this context, in the Marmara Region, renewable energy sources can be used in livestock enterprises that are far from settlements and the electricity grid. A fan-pad system will be applied to ensure appropriate environmental conditions in animal barns. For this reason, an evaporative fan-pad system was designed in animal barns in Tekirdağ conditions. There are 70 cattle in the barn where the project will be carried out and the dimensions of the barn are 10.4 x 46.1 (479.4 m^2). To cool this barn, a pad area of 19.2 m^2 is required and a 0.2 kWh circulation pump is required to cycle the water to evaporate. The number of aspirators to be used in ventilation is 9 and their efficiency is 9500 m^3h^{-1}. Electricity consumption is 0.75 kWh. The power value calculated in the ventilation and cooling system is 6.95 kWh. However, solar and wind energy production systems change throughout the day. For this reason, a system of 9-10 kWh, which is 25-30% more than the calculated power, should be installed. 5 kWh of this should be solar panels and 5 kWh should be wind turbine systems. With a 5 kWh solar panel system, 10.035 kWh of electrical energy can be obtained per year, and with a wind turbine system, 9.125 kWh of electrical energy can be obtained per year. With this hybrid system, a total of 19.160 kWh of electrical energy can be produced annually. This will prevent the release of 18.010,4 kg (19.160 x 0.94 = 18.010,4 kg) of CO_2 gas into nature. A 10 kWh hybrid energy system in Tekirdağ conditions prevents significant CO_2 gas emissions into the atmosphere.
Keywords: *Hybrid energy, Animal barn, Wind turbine, Solar panel, Fan-pad system*

INTRODUCTION

One of the most important problems the world faces in the 21st century is the increasingly growing demand for energy. The main factors behind this are population growth, urbanization, industrialization, and the advancement of technology. Today, the majority of energy needs are met by fossil fuels. The widespread use of fossil fuels has been driven by their economic affordability, rapid advancements in production systems, and their widespread availability. The use of fossil fuels, starting with coal and later followed by a significant increase in the consumption of oil and natural gas, has risen dramatically. Today, the continuous rise in the price of fossil-based energy, transportation issues, the tendency of reserves to decrease, and the fact that they will eventually be exhausted are seen as major problems (Yılmaz, 2012). Additionally, the increase in CO_2 emissions, leading to global warming and a heightened risk of climate change, has increased the demand for renewable energy in energy policies and highlighted the importance of this issue. "Renewable energy source can be defined as an energy source that can be naturally replenished the very next day as part of nature's own evolution." Renewable energy sources, considered as alternatives to fossil energy sources, are environmentally friendly, continuously available in nature, and provide clean and sustainable energy (Yılmaz and Can-Öziç, 2018).

The use of renewable energy sources alongside other energy sources, forming hybrid systems, provides a significant advantage. The fact that solar energy is only available during the daytime and wind is not constant limits the independent use of these systems. As a solution, it is possible to install the system with high capacity and store energy in batteries, allowing the stored energy to be used when the system is not generating power (Türkdoğan et al., 2020). Renewable energy sources can diversify a country's energy resources and enhance its energy security. By utilizing renewable energy sources, energy imports can be reduced. In this way, the country's energy sector independence is increased, and by reducing the current account deficit in the national budget, it contributes to economic development (Koçaslan, 2010).

The sole use of wind and solar energy systems may pose challenges in terms of energy supply security. For this reason, their use in a hybrid manner can ensure uninterrupted energy supply. Additionally, there is a higher potential for wind energy during the winter months and a higher potential for solar energy during the summer months. A wind-solar hybrid power plant is an important alternative for sustainable energy supply. These types of hybrid energy systems generally have better efficiency, economic benefits, and environmental values compared to independent systems (Rezai et al., 2018).

It is stated that solar energy systems and wind energy systems can be used in the agricultural production and livestock sectors (Yüksel and Türkboyları, 2018; Türkboyları and Yüksel, 2024a). The energy needs in the livestock sector are even more significant because they are located in areas far from the electricity grid. Because livestock enterprises must be established far from settlement centers. It will be easier to meet the energy needs of animal barns located in off-grid and remote areas using solar-wind hybrid systems. This is because these systems complement each other in the summer and winter months (Türkdoğan et al., 2020).

Livestock farming is an agricultural production sector that requires significant effort both globally and in Turkey. It is an economically and socially significant sector that provides animal proteins needed by people, such as meat, milk, yogurt, and butter. In other words, it ensures the balanced intake of calories, proteins, fats, and carbohydrates that the body needs for a balanced diet (Karacan, 2017). Since Turkey's meat production, which is a source of animal protein, is not sufficient, meat is occasionally imported from other countries. Turkey's per capita meat consumption is seen to be low compared to developed countries. According to 2019 data, Turkey's per capita meat consumption is 36.1 kg (beef 13.6 kg, poultry 21.0 kg, sheep 1.5 kg) (BESD-BİR, 2023).

Considering the population growth in Turkey and the world, increasing the quantity and quality of animal and plant products in parallel is also an important issue. When evaluating meat and milk production in the world, it is observed that cattle alone account for 90% of milk production and 25% of meat production (Şahin and Ulutaş, 2011; Uğur, 2014).

This study focuses on meeting the energy needs of animal barns, which are part of agricultural production structures, through solar-wind hybrid energy systems. Providing suitable environmental conditions in animal barns is crucial for increasing productivity. According to the law, barns must be constructed outside of residential areas. This requirement hinders access to the electricity grid.

Therefore, a project has been developed to meet the electricity needs of the fan-pad system used to provide environmental conditions in the barn. Additionally, the implementation of the proposed energy system will significantly reduce the emission of CO_2 gases into the atmosphere.

Environmental Conditions in Animal Barns

Agricultural production is an important sector in our country, as well as in the world, for the nutrition of both humans and animals. In many of the leading agricultural countries, the proportion of livestock activities within agricultural production is at a significantly high level. However, when evaluated in terms of ecology, geography, and farming culture, Turkey is a country that is quite suitable for livestock farming. Despite all these positive conditions, animal production in Turkey ranks after plant production (Ören and Bahadır, 2005; Turan et al., 2017).

The genetic structure of animals and the environmental factors they are exposed to are two elements that affect productivity in animal production. In addition to being genetically high-yielding, an animal must have an environment that provides the necessary conditions to express its genetic potential in terms of productivity (Tugay and Bakır, 2009). Suitable environmental conditions in the barn can only be achieved through proper ventilation of the barn. Ventilation in animal barns is achieved in two ways: naturally and mechanically. Natural ventilation can be defined as the process by which the air inside the barn is replaced with outside air due to the temperature difference between the outside and inside air, as well as the humidity and wind effects. The natural ventilation system does not operate during times of high and stagnant air temperatures. In this case, mechanical ventilation should be implemented to prevent a decrease in productivity in animals. Mechanical ventilation requires electrical energy.

Inadequate ventilation slows down the growth of animals and reduces their productivity. At the same time, it also increases the risk of diseases.

With ventilation, the air inside the barn is refreshed, ensuring the climatic and chemical environmental conditions within the barn. Temperature and humidity inside the barn are generated through the animals' skin and respiration. Therefore, the temperature inside the barn increases, and the humidity level rises. The dust from the roughage and straw given to the animals affects the physical properties of the air. The gases (CO_2, NH_3, and H_2S) produced as a result of the metabolism of the animals in the barn, along with microorganisms, elevate the properties of the air inside the barn to undesirable levels. The negative physical and chemical properties of the air can only be transformed into the desired condition through mechanical ventilation (Yüksel and Şişman, 2015).

The energy required for the ventilation system can be provided in remote areas and rural regions by utilizing solar-wind hybrid energy systems, through photovoltaic panels and wind turbines. In areas where grid electricity is available, the same systems can be used during power outages or to reduce energy and consumption costs.

The Potential of Turkey's Solar-Wind Hybrid Renewable Energy Resources

Due to its geographical location, Turkey has a much higher potential for solar energy and coastal areas for wind energy compared to many countries. However, these energy systems are intermittent, fluctuating, and complementary in nature. During the summer months, when the sunlight is very bright and strong, wind energy is low. In the winter months, when solar energy is low, wind speeds are high. The efficiency of energy generation from wind and solar energy systems varies from year to year and from day to day (Özdamar et al., 2005). In other words, solar energy is utilized when wind speeds are low, and wind energy is harnessed when solar energy is insufficient. Thus, the continuity of energy production in the system is ensured. The solar-wind hybrid power system is shown in Figure 1 (Atik and Sekin, 2022).

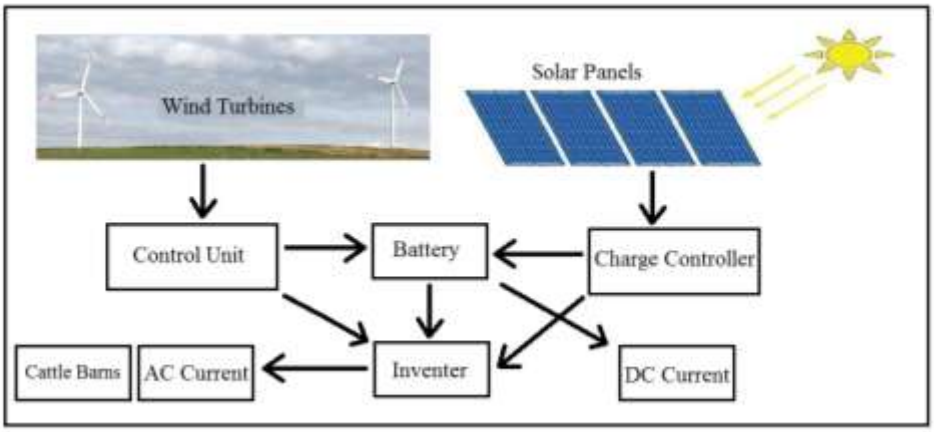


Figure 1. Off-grid solar-wind hybrid power system diagram (Atik and Sekin, 2022)

Hybrid systems can be established as grid-connected (on-grid) or independent from the grid (off-grid). In an on-grid system, the electricity generated is not stored in batteries but is directly transferred to the grid. This generated energy can be connected to the central grid through inverters (Wang et al., 2015). The most important reason for installing this system is its ease of installation. Additionally, the absence of a requirement to use batteries eliminates the cost of energy storage. Since the system is connected to the grid, if the generated energy is not sufficient, energy is directly drawn from the grid to meet the energy needs.

In animal barns, off-grid systems, which are independent of the grid, provide the necessary energy in situations where there is no access to the electricity grid. The requirement for animal barns to be located in rural areas can also create challenges in accessing electrical energy. Even if the barn is close to the grid, a renewable energy-based system can be installed to provide power during outages or to reduce the energy costs of the operation. Since the system is off-grid, it must be supported with batteries. When the hybrid system is unable to generate enough electricity, the energy needs are met by the batteries.

MATERIAL AND METHOD

Methods

The study was conducted in Tekirdağ Central, located in the Thrace peninsula of Turkey's European continent. The Thrace peninsula is situated in the northwest of Turkey, between 26°-29° eastern longitudes and 40°-42° northern latitudes. Tekirdağ is located on a peninsula surrounded by the Black Sea, the Bosphorus, the Sea of Marmara, the Dardanelles, and the Aegean Sea. Its area is 6.313 km². Tekirdağ has a topographically undulating surface. It has a semi-humid climate, with dry summers and rainy autumn, winter, and spring seasons. It is windy in summer and winter (Anonymous, 2023a). According to Turkey's Solar Energy Potential Atlas (GEPA) and Wind Energy Potential Atlas (REPA), Tekirdağ and the Marmara coasts in our country have significant solar and wind energy potential (Anonymous, 2024a; Karık et al., 2017; Tunus, 2019). Therefore, Tekirdağ is highly advantageous in terms of generating electricity from solar energy in the summer and wind energy in the winter.

A project has been developed in Tekirdağ to utilize solar and wind energy in a hybrid manner for ventilation and cooling purposes in beef cattle barns. A mechanical ventilation and cooling system has been designed for a 70-head beef cattle barn where the project will be implemented. The dimensions of the project barn are 10.4x46.1=479.4 m² (Yüksel and Şişman, 2015).

For the cattle barn, which has dimensions of 10.4x46.1=479.4 m² and houses 70 cows, a project will be designed to include ventilation fans, cooling pads (wet pads), and energy production systems using solar panels and wind turbines.

Design of Solar-Wind Hybrid Energy Systems for Animal Barns

Turkey, situated between two continents and composed of two peninsulas, is far more advantageous in terms of solar and wind energy compared to many other countries. In this context, utilizing renewable energy sources in plant and animal production helps reduce production costs. Despite the high initial investment cost of these production systems, the absence of raw material costs, low maintenance expenses, and their long lifespan contribute to reducing energy production costs as well. Studies have shown that solar and wind energy sources can be used economically in livestock enterprises (Yüksel and Türkboyları, 2018; Orhan and Şahin, 2022).

The solar panel system consists of solar cells that convert sunlight directly into electrical energy. They are made from materials such as silicon, which exhibits semiconductor properties. To use silicon as a solar cell, it can be doped with phosphorus and aluminum. The use of inorganic-based solar cells is more common, and their efficiencies range from 15% to 20% (Grätzel, 2009).

Wind turbines are the fundamental components of wind energy plants. They are machines that convert the kinetic energy of moving air into electrical energy. The wind turbine generates electrical energy between the lowest (2-4 ms^{-1}) and the highest (25-35 ms^{-1}) wind speeds. The maximum wind energy from the system can be obtained at the nominal wind speed (10-15 ms^{-1}) (Anonymous, 2023b).

In the structure of solar and wind turbine hybrid systems, depending on the application, there may be solar panels, a wind turbine, accumulators, a charge regulator, an inverter, various circuits, fuses, and a control center (Toprak, 2011; Şenel and Koç, 2015). In this system, the solar panel and wind turbine system needed for power are used as energy sources. There is a mismatch between the supply and demand of solar and wind energy. In other words, due to the inability to produce energy when needed, batteries are connected to the system. The battery group is used as a backup system. The batteries provide energy to the system when there is no sunlight or wind. The charge regulator added to the system prevents the batteries from being overcharged and discharged. By using a charge regulator, the lifespan of the batteries is extended, which also reduces their depreciation values. Electric devices and equipment such as lighting and motors used in Turkey are produced for alternating current. Additionally, if the system is to supply electricity to the grid, it must include an inverter. The inverter converts the direct current (DC) obtained from hybrid solar and wind energy systems into alternating current (AC).

Ventilation and Cooling Systems in Animal Barns

In the cooling of animal barns, a ventilation system with a fan-pad system can be used (Figure 2) (Yüksel and Yüksel-Türkboyları, 2018; Yüksel and Şişman, 2015).

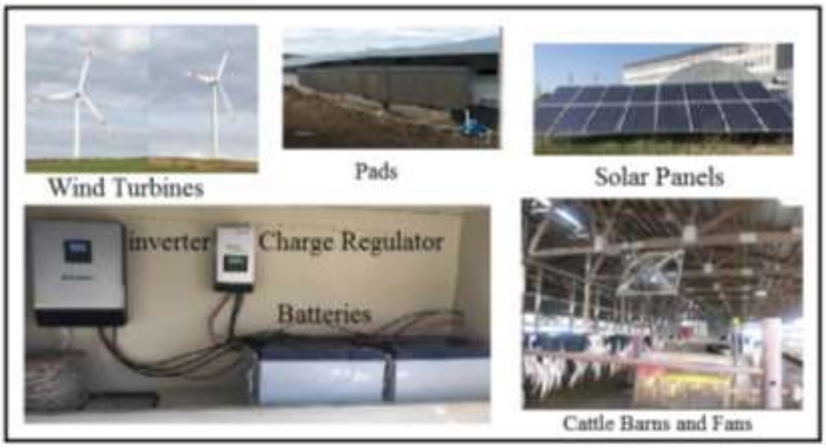


Figure 2. Cooling systems in animal barns using a solar-wind hybrid energy system.

The components of the cooling system include fans, wet pads, circulation pumps, water transport pipes, and a water source. In cooling the animal barn with water, the energy that the water absorbs from the environment, specifically from the air, during evaporation is utilized. Since water absorbs latent heat from the air's sensible heat during evaporation, it also reduces the temperature of the air entering the barn through ventilation (Boyacı et al., 2012; Yüksel and Şişman, 2015; Yüksel and Türkboyları, 2018). The energy required for the cooling system is supplied by the solar-wind hybrid energy system. The system is arranged such that the pads are placed on the opposite side of the walls where the fans are installed inside the barn. When the fans start to operate, the air inside the barn is expelled, creating a low pressure within the barn. The humid and cooled air enters the barn through the openings of the pad on the opposite wall, lowering the temperature of the air inside the barn.
The amount of ventilation in an animal barn can be calculated in two ways. The first method can be calculated based on the internal volume of the barn, and the second method can be calculated according to the number of animals in the barn. The more suitable of these calculations is the one based on the number of animals.
In the conditions of Tekirdağ, ventilation is needed to some extent in every season. The increased need for ventilation in hot seasons is less than in cold seasons. Ventilation should be combined with cooling during June, July, and August when the outside air temperature rises above 22-24°C (Yüksel and Şişman, 2015; Yüksel and Türkboyları, 2018).

RESULTS AND DISCUSSION

Design of Fan-Pad Systems to Be Used in Animal Barns

To design the wet pad to be used for cooling the barn, the dimensions of the barn must be known. The width of the barn for the wet pad project is 10.1 m, and the length is 46.1 m, designed for 70 beef cattle (Yüksel and Şişman, 2015).
In the design of wet pads, 1 m² of wet pad area is required for every 25 m² of floor area (A_1) in the animal barn. For a cattle barn with a floor area of 479.4 m² (A_b) (10.1 x 46.1 m), the required pad area (A_p) can be calculated as follows (Equation 1).

$$A_p = A_b/A_1 = 479.4/25 = 19.2\ m^2 \tag{1}$$

The daily water requirement of the wet pad varies depending on the air temperature, but on hot days, it is around 30-40 L per 1 m² of pad area (Bucklin et al., 1993). The water needed for cooling the pad is taken from the water source with a circulation pump and delivered to the pad. The circulation pump with a power of 0.2 kWh is sufficient.

There are various factors that affect the ventilation rate in animal barns. These include geographical location, seasons, and most importantly, the number of animals in the barn. For large livestock with a live weight of 500 kg, the ventilation rate per animal can be considered as 400 m^3h^{-1}. This value is for the northern regions of Turkey. However, since the average weight of fattening cattle is 700-750 kg, the ventilation rate should be taken as 600 m^3h^{-1} per animal (Q_1) (Türkboyları and Yüksel, 2024b). Accordingly, the ventilation amount (Q_t) based on the number of animals in the barn (n) can be calculated as follows (Equation 2).

$$Q_t = Q_1 \times n = 600 \times 70 = 42000\, m^3h^{-1} \tag{2}$$

The exhaust fans in the barn can be operated in varying numbers based on the needs. In animal barns, exhaust fans with a diameter of 60 cm that produce low noise should be preferred. Their power is 0.75 kWh, and their efficiency (Q_a) is 9500 m^3h^{-1} (Anonymous, 2024b).

The number of exhaust fans (n_a) to be used in the system can be calculated using Equation 3.

$$n_a = Q_t/Q_a = 42000/9500 = 4.4{\sim}5 \text{ piece} \tag{3}$$

The number of exhaust fans to be used in the barn is higher due to the length of the barn. There should be a distance of 15-20 meters between the exhaust fans for proper air movement within the barn. In this barn, two exhaust fans are needed at 15-17 meters and two more at 35-36 meters, making a total of four additional fans required. Thus, the system should have a total of 9 exhaust fans (Yüksel and Türkboyları, 2018).

The total energy requirement of the system can be calculated using Equation 4.

$$0.75 \times 9 + 0.2 = 6.95\, kWh \tag{4}$$

Approximately a 7 kWh solar or wind energy system is required. However, if the systems are considered separately, the solar panel system cannot produce enough energy at night, on cloudy days, and during rainy weather. In the wind turbine system, fluctuating wind speeds or a complete lack of wind can create issues for energy production (Tunus, 2019). Therefore, the systems are kept 25-30% larger than the calculated value. The designed system should be converted into a 9-10 kWh system. Therefore, the total energy production of the solar panel and wind turbine systems should be considered as 10 kWh. The energy system should be designed as a 5 kWh solar panel system and a 5 kWh wind turbine system.

Impact of Solar-Wind Hybrid Energy System on CO_2 Emissions

Countries' electricity generation has primarily started with non-renewable energy sources, particularly coal. Later, various renewable energy sources such as natural gas, hydropower, geothermal, biomass, and nuclear energy have also been integrated into the system. The non-renewable energy sources that are largely used in electricity generation pollute the environment with various waste products. Due to the negative effects of these waste products on the climate, there has been a shift towards renewable energy sources as clean energy alternatives. Among these sources, solar and wind energy play a leading role.

Coal is an important source of electricity generation in Turkey. Approximately 21% of electricity production is derived from coal (Orhan and Şahin, 2022). In thermal power plants used for electricity production, coal is used as fuel, resulting in significant CO_2 emissions into the environment. According to the Carbon Neutral Charitable Fund (CNCF, 2023), the amount of CO_2 released into the atmosphere by thermal power plants to produce 1 kWh of electrical energy is approximately 0.94 kg. Accordingly, the amount of CO_2 prevented from being released into the atmosphere can be calculated by multiplying the energy amount in kWh produced from solar and wind energy systems by 0.94. Thus, the use of renewable energy sources helps to protect nature as well.

It has been determined that a 10 kWh energy system is needed for the project. In other words, the hybrid system should produce 10 kW of electrical energy per hour. Since the system is designed as a hybrid, it will consist of a 5 kWh wind turbine system and a 5 kWh solar panel system. Both energy systems may operate for longer or shorter periods on certain days. In other words, there may be fluctuations and interruptions in energy production. When the systems operate efficiently for extended periods, the excess energy is sent to the grid in an on-grid system, and stored in the battery in an off-grid system. In times when energy production is insufficient, the stored energy is utilized. It can be assumed that the wind turbine energy system operates for approximately 5 hours a day (Türkboyları and Yüksel, 2024b). Given that the power of the wind turbine is 5 kWh and its daily operating time is 5 hours, the amount of energy it can produce annually is calculated using Equation 5.

$$5\ kWh \times 5\ hours\ day^{-1} \times 365\ days\ year^{-1} = 9.125\ kWh \tag{5}$$

With the wind turbine energy system, approximately 8.577,5 kg (9.125×0.94=8.577,5kg) of CO_2 emissions into the atmosphere are prevented.

To determine the relationship between CO_2 emissions and the solar panel energy system, which is the other power source to be used in the trial, it is necessary to know the sunshine durations of Tekirdağ province (Anonymous, 2024c). These values are provided in Table 1.

Table 1. Sunshine duration of Tekirdağ province (hours) (Anonymous, 2024c)

Months	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Total**
Sunshine duration	2.7	3.3	4.2	5.8	7.3	8.6	9.4	8.5	6.8	4.6	3.2	2.5	66.9

In calculating the amount of electrical energy that can be produced in Tekirdağ with the projected 5 kWh photovoltaic panel system, the total average daily sunshine hours on a monthly basis can be calculated by multiplying the monthly number of days by the power of the panel system (Equation 6).

$$66.9\ hours\ month^{-1} \times 30\ days\ month^{-1} \times 5\ kWh = 10.035\ kWh\ year^{-1} \tag{6}$$

With the installation of a 5 kWh solar panel system, 10.035 kWh of electricity can be generated annually. This produced electricity can prevent the release of 9.432,9 kg (10.035 x 0.94 = 9.432,9 kg) of CO_2 into the atmosphere.

A hybrid renewable energy system, intended for agricultural use in animal barns, can significantly prevent CO_2 emissions. With a 10 kWh hybrid energy system consisting of solar panels and a wind turbine energy system, 19.160 kWh of electricity can be produced annually (9.125 kWh from the wind turbine and 10.035 kWh from the solar panel system). With the produced electricity, 18.010,4 kg of CO_2 emissions can be prevented annually. With the use of renewable hybrid energy sources, the release of harmful waste gases caused by fossil fuels in energy production is significantly prevented from being released into the atmosphere.

CONCLUSION

In Turkey, energy consumption is increasing primarily due to population growth, along with various other reasons. The increasing energy demand also poses a significant burden on the national economy, contributing to the budget deficit. In the livestock sector, energy demand constitutes the largest expense item. This energy is used for ventilation, cooling, and heating (Arslan, 2024).

Renewable energy sources can be utilized to provide economic benefits to the national economy and livestock enterprises. Because the Marmara region, where the project is carried out, is one of the areas with high solar and wind energy potential. However, these energy sources are not being utilized sufficiently. The reason for this is the high initial investment cost of solar and wind energy systems (Emiroğlu et al., 2021).

In the Marmara region, electricity can be generated and utilized in animal barns using solar and wind hybrid energy systems. In addition to providing energy from renewable sources to agricultural enterprises in rural areas, a significant amount of CO_2 emissions into the atmosphere can be prevented.

Animal barns should be ventilated and cooled according to the season to obtain high yields. The energy costs of the operation can be covered by utilizing the energy obtained from the designed hybrid systems. The surplus energy produced in the facility can be used in other units of the operation or supplied to the national electricity grid, providing financial benefits to the operation (Türkboyları and Yüksel, 2024b).

With the use of renewable energy sources in agriculture, CO_2 production, which contributes to climate change, can be reduced, thereby slowing down the pace of climate change.

REFERENCES

Anonymous (2023a). https://tekirdag.ktb.gov.tr/TR-75726/genel-bilgiler.html (Accessed date: 15.06.2023)

Anonymous (2023b). https://enerji.gov.tr/eigm-yenilenebilir-enerji-kaynaklar-ruzgar (Accessed date: 02.04.2023)

Anonymous (2024a). https://enerji.gov.tr/eigm-yenilenebilir-enerji-kaynaklar-gunes (Accessed date: 11.07.2024)

Anonymous (2024b). https://www.hvacturk.com/urun/ayas-60-cm-sanayi-tipi-aspirator (Accessed date: 11.07.2024)

Anonymous (2024c). https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=H&m=TEKIRDAG (Accessed date: 12.07.2024)

Arslan, A. (2024). Analysis of the energy efficiency of poultry houses in Turkiye. Black Sea Journal of Engineering and Science, 7(2):277-297.

Atik, İ. and Sekin, A., 2022. Hibrit yenilenebilir enerji sistem tasarımı: Balıkesir örneği. Dicle Üniversitesi Mühendislik Fakültesi Mühendislik Dergisi, 13(3):517-529.

BESD-BİR (2023). https://besd-bir.org/assets/uploaded/Tr-kisi-basina-turlere-gore-et-tuketimi.pdf (Accessed date: 17.08.2023)

Boyacı, S., A. Akyüz, S. Gençoğlan, Ş. Baydar (2012). Etlik piliç kümeslerinin fan-ped sistemiyle serinletilmesi. II. Ulusal Sulama ve Tarımsal Yapılar Sempozyumu, 24-25 Mayıs:949-954, İzmir.

Bucklin R.A., R.W. Henley, D.B. McConnel (1993). Fan and pad greenhouse evaporative cooling systems. University of Florida, Florida Cooperative Extension Service, Circular 1135.

CNCF (Carbon Neutral Charitable Fund) (2023). https://carbonpositiveaustralia.org.au/calculate/ (Accessed date: 01.08.2023)

Emiroğlu, M.F., A. Aybek, H. Kuzu (2021). İki farklı fotovoltaik (PV) enerji sisteminin farklı hayvancılık işletmelerinde kullanımının değerlendirilmesi. Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 26(3):808-820.

Grätzel, M. (2009). Recent advances in sensitized mesoscopic solar cells. Accounts of Chemical Research, 42(11):1788-1798.

Karacan, R. (2017). Türkiye'de kırmızı et talebinin, beyaz et tüketimi ve gelir dağılımı açısından değerlendirilmesi. Finans Politik ve Ekonomik Yorumlar, 54(630):67-73.

Karık, F., A. Sözen, M.M. İzgeç (2017). Rüzgar gücü tahminlerinin önemi: Türkiye elektrik piyasasında bir uygulama. Politeknik Dergisi, 20(4):851-861.

Koçaslan, G. (2010). Sürdürülebilir kalkınma hedefi çerçevesinde Türkiye'nin rüzgar enerjisi potansiyelinin yeri ve önemi. Sosyal Bilimler Dergisi, 1:53-61.

Orhan, N., S. Şahin (2022). Bir besi çitliğinde güneş enerji sisteminin uygulanması ve ekonomik analizi. Türk Tarım ve Doğa Bilimleri Dergisi, 9(1):33-40.

Ören, M.N., B. Bahadır (2005). Türkiye'de ve OECD ülkelerinde hayvansal ürün politikaları ve bu politikalar sonucu ortaya çıkan transferler. Hayvansal Üretim Dergisi, 46(1):1-7.

Özdamar, A., N. Özbalta, A. Akın, E.D. Yıldırım (2005). An application of a combined wind and solar energy system İzmir. Renewable and Sustainable Energy Reviews, 9(6):624-637.

Rezai, M., A. Mostafaeipour, M. Qolipour, R. Tavakkoli-Moghaddam (2018). Investigation of the optimal location design of a hybrid wind-solar plant: A case study. International Journal of Hydrogen Energy, 43(1):100-114.

Şahin, A., Z. Ulutaş (2011). Tahirova Tarım İşletmesinde yetiştirilen siyah alaca ineklerin süt ve döl verim özelliklerini etkileyen bazı çevresel faktörler. Anadolu Tarım Bilimleri Dergisi, 26(2):156-168.

Şenel, M.C., E. Koç (2015). Dünya'da ve Türkiye'de rüzgar enerjisi durumu-genel değerlendirme. Mühendis ve Makine, 56(663):46-56.

Toprak, A. (2011). Elektrik üretimi için düşük güçlü rüzgar enerji sistemi tasarımı. Selçuk Üniversitesi, Fen Bilimleri Enstitüsü Yüksek Lisans Tezi, 82p., Konya.

Tugay, A., G. Bakır (2009). Giresun yöresindeki süt sığırcılığı işletmelerinin yapısal özellikleri. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 40(1):37-47.

Tunus, O. (2019). Bursa'da yenilenebilir enerji kaynakları ile elektrik üretim potansiyelinin ekonomik analizi. Bursa Uludağ Üniversitesi Sosyal Bilimler Enstitüsü Yayınlanmamış Yüksek Lisans Tezi, 141p., Bursa.

Turan, Z., D. Şanver, K. Öztürk (2017). Türkiye'de hayvancılık sektöründen süt inekçiliğinin önemi ve yurtiçi hasılaya katkısı ve dış ülkelerle karşılaştırılması. Ömer Halisdemir Üni. İktisadi ve İdari Bilimler Fakültesi Dergisi, 10(3):60-74.

Türkboyları, E., A.N. Yüksel (2024a). Meeting the energy needs of poultry houses with wind turbine system under Tekirdağ conditions and its environmental effects. Black Sea Journal of Engineering and Science (BSJ Eng Sci), 7(3):594-600.

Türkboyları, E., A.N. Yüksel (2024b). Rüzgar türbinlerinin tarımsal amaçla hayvan barınaklarında enerji kaynağı olarak kullanılma olanakları. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 7(2):610-621.

Türkdoğan, S., M.T. Mercan, T. Çatal (2020). Şebekeden bağımsız hibrit enerji sistemleri kullanılarak 40 hanelik bir topluluğun elektrik ve termal yük ihtiyacının karşılanması: Teknik ve ekonomik analizleri. Avrupa Bilim ve Teknoloji Dergisi, 18:476-485.

Uğur, F. (2014). Sığır Yetiştirme. Çanakkale 18 Mart Üniversitesi Yayınları No:117, p.90-100. ISBN:978-605-422-36-0.

Wang, H., E. Abdollahi, R. Lahdelma, W. Jiao, Z. Zhou (2015). Modelling and optimization of the smart hybrid renewable energy for communities (SHREC). Renewable Energy, 84:114-123.

Yılmaz, M. (2012). Türkiye'nin enerji potansiyeli ve yenilenebilir enerji kaynaklarının elektrik enerjisi üretimi açısından önemi. Ankara Üniversitesi Çevrebilimleri Dergisi, 4(2):33-54.

Yılmaz, E.A., H. Can-Öziç (2018). Türkiye'nin yenilenebilir enerji potansiyeli ve gelecek hedefleri. Ordu Üniversitesi Sosyal Bilimler Araştırmaları Dergisi, 8(3):525-535.

Yüksel, A.N., E. Yüksel-Türkboyları (2018). Using the photovoltaic cells for ventilation and cooling of the animal barns. 1st International and 14th National Congress on Agricultural Structures and Irrigation, 26-28 September 2018 (ICASI-2018), Antalya-Turkey:49-55.

Yüksel, A.N., C.B. Şişman (2015). Hayvan Barınaklarının Planlanması. Hasad Yayıncılık, İstanbul.

DETERMINATION OF THE VOLUME OF TRANSPORT WORK WHEN TRANSPORTING LETTUCE TO THE VEGETABLE EXCHANGE FRUIT PRODUCED IN SMALL FARMS

Stoian FILIPOV[1], Kostadin KOSTADINOV[1], Ivan ZAHARIEV[2]*

[1] Agricultural University - Plovdiv, 12 Mendeleev Blvd, Dept of "Horiculture", Plovdiv, Bulgaria;
[2] Agricultural University - Plovdiv, 12 Mendeleev Blvd, Dept of "Mechanization of agriculture", Plovdiv, Bulgaria.

** Corresponding author e-mail: stoyanf@abv.bg*

ABSTRACT

The article presents the results of the study of the transport scheme and the routes from the vegetable market in the village of Plodovitovo to the villages located up to 25 km from it. The small farmers in each of the villages have been identified, their planted areas with lettuce expected to appear on the market at the same time. The mass of one piece of salad was measured m_z = 684g, the mass of one transport package with salad m_k = 7.368 kg. The average yield of 1da - Q_{cc} = 1694.3 kg/da was determined. Based on the determined routes and quantities for daily transport from each village to the vegetable exchange, the transport work for each route was calculated. A recommendation was made to monitor stock in the stock exchange and the market situation in order not to prepare production that would remain and thus generate losses for everyone in the chain.

Keywords: Salad, transport work, means of transport, routes of movement

INTRODUCTION

Vegetable production is a branch whose products occupy a significant part of the country's food supply. This determines the important place of vegetable production in the national economy. This industry is not only economically important for feeding the population, but also a way of livelihood and life for a significant part of it.

Nowadays, lettuce is widely consumed worldwide due to its beneficial health properties. Lettuce is rich in fiber, the consumption of which regulates the gastrointestinal tract and reduces the risk of colon cancer. In addition, lettuce is vegetable rich in natural antioxidants, such as quercetin, kaempferol, luteolin, and ascorbic acid (Llorach, Martínez-Sánchez, Tomás-Barberán, Gil, & Ferreres, 2008; Pereira, Rodrigues, & Ramalhosa, 2013).

Agricultural products are grown in farms of different sizes. Small farms in the country, as part of the European Union, are of interest. Small farms continue to be a key element of Union agriculture, as they play a vital role in supporting rural employment and contributing to territorial development (https://agriculture.ec.europa.eu …). Cultivation of areas planted with lettuce in the Republic of Bulgaria is in two ways: "greenhouse cultivation" and "field cultivation".

Greenhouse cultivation is accompanied by additional costs for the farmer for materials, labor and heating of the greenhouses. Lettuce grown in greenhouses reach marketable size by the 3rd week of February. The last greenhouse salads are available for sale until the beginning of April. On the other hand, the production that came to the market earlier has a higher selling price and before the direct vegetable competitors of the salads - tomatoes and cucumbers. This is confirmed by the fact that the Bulgarian sharply reduces the consumption of lettuce at the moment when the selling prices of tomatoes and cucumbers fall to the levels of his purchasing power.

The harvesting period of the salads ready for the market is 20-25 days depending on the climatic conditions.

The production from the field cultivation begins to be offered on the market with some overlap with that from the greenhouse production (end of March - beginning of April). This means that at some point the last batches of greenhouse lettuces and the new production from the fields will be delivered to the exchange at the same time. This overlapping period varies in duration and calendar time of occurrence. It mainly depends on two factors:

– climatic conditions that change every year;

– the financial and time estimates of individual producers of greenhouse lettuce.

Another indisputable fact that needs to be taken into account is the yield fluctuations within each farm.

The import factor of lettuce from abroad has its influence on the volume of transport.

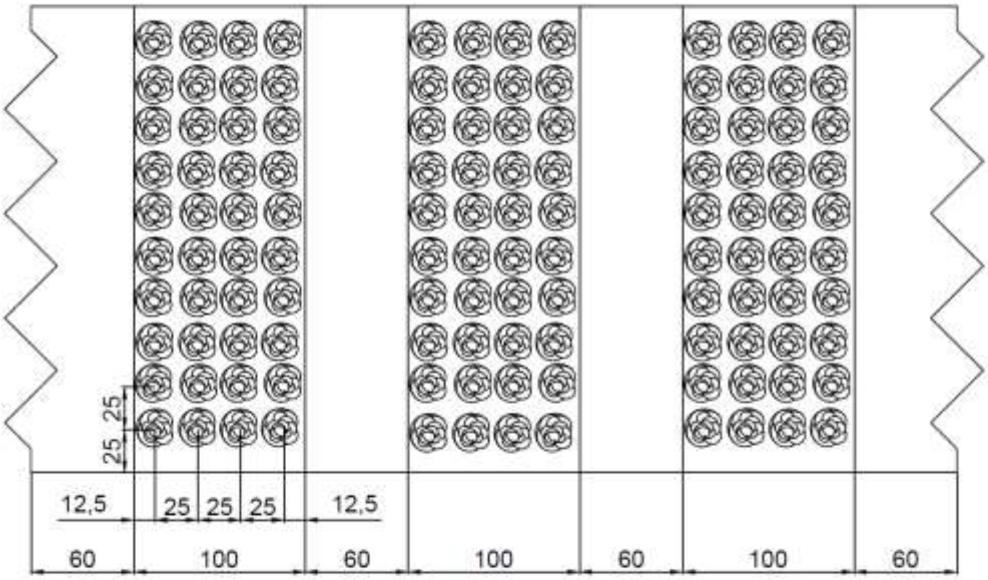


Figure 1. Lettuce planting scheme 25x25 cm

Transport is a major structure-determining branch (Barbov et al., 1993; Vasilev, 2004; Directives of the EU regarding transport policy, by types of transport; Tsankov, 1994; Zahariev, 2023) According to Radkov (2005) the factors that have a direct or indirect influence on the state and development of transport can be generally defined as internal and external. External factors can be conditionally divided into global, regional and external to the transport system within the country. Internal ones in the context of international integration are of national and local importance.

According to https://eurocode.bg/... the volumetric weight for salads is γ=5.0 kN/m3 = ~ 500 kg/m3 in free bulk (volumetric weight of loosely (disorderly) bulked or accumulated in large quantities of products).

When preparing lettuce for commercial purposes, 8-10 pieces are arranged in the transport package, depending on the variety and size.

Due to the wide distribution of banana packaging - cartons and their reusability are also used as transport packaging for lettuce. To protect the carton from moisture damage, a plastic bag is placed inside the carton and then the salads are arranged.

According to ORDER No. 16 of 31.05.1999 on physiological norms and rules for manual work with weights, the weight of the load does not exceed 15 kg for women and 50 kg for men for a single load when lifting, supporting, moving and carrying a distance of 2 m and 4,000 kg for women and 10,000 kg for men - total for change (https://www.gli.government.bg …; Zahariev, I. 2023; Zahariev, I. 2024).

The location of the wholesale market for fruits and vegetables near the village of Plodovitovo and the road connections with neighboring settlements are shown in fig. 2.

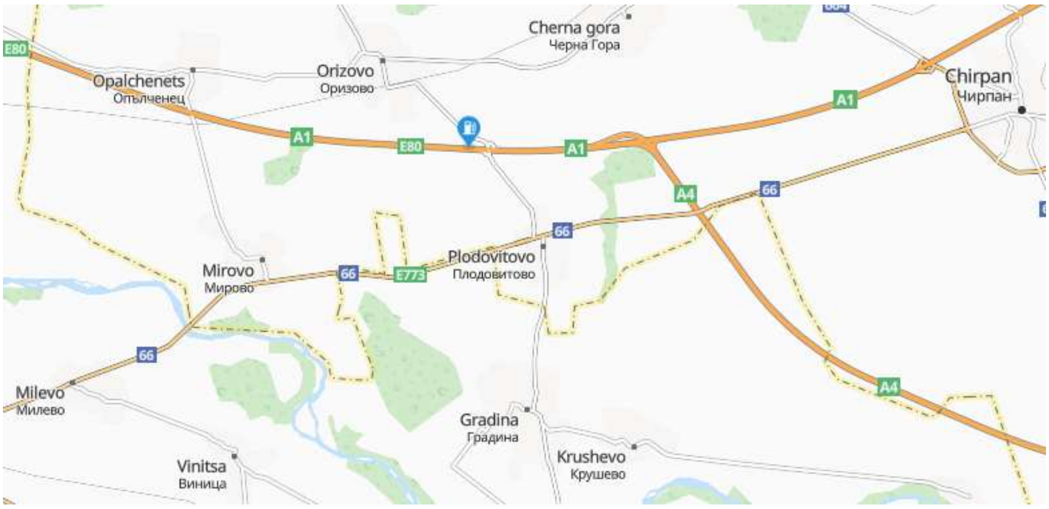


Figure 2. Location of Plodovitovo village (https://www.viamichelin.com/ …)

According to data posted on the website https://titul.bg/... Plodovitovo Vegetable Exchange is open 24 hours a day on all days of the week. According to data from the Administrative Register of the Republic of Bulgaria (https://iisda.government.bg …) the village of Plodovitovo belongs to Bratya Daskalovi municipality, Stara Zagora district. Highway A1 (Sofia-Burgas) passes by the village, which branches off to the east and there begins highway A4 (to Svilengrad and the border with the Republic of Turkey). The national road 66 Plovdiv - Stara Zagora passes through the village. The surrounding settlements around the village of Plodovitovo are connected by an asphalt road.

MATERIAL AND METHOD

The settlements within a radius of 25 km from the village of Plodovitovo are determined using electronic road maps. A diagram of the location of the villages in relation to the village of Plodovitovo is drawn and the transport connections between them are determined. The distance and travel time from Plodovitovo to each settlement are recorded in tabular form. On the basis of the collected data, the transport scheme, the carrying capacity of the means of transport and the ready quantities prepared for loading by the various manufacturers, the transport routes are made. After determining the routes, the distance and travel time from Plodovitovo to the most extreme point is calculated, taking into account that the vehicle moves without loads and along the shortest distance. On the way back, it starts from the farthest point through the intermediate stops to Plodovitovo:

$$L_{TM} = L_{P\_B1} + \sum_{i=1}^{n} L_{Bi} + L_{P\_B2} \quad (1)$$

where: L_{P_B1} is the shortest distance from Plodovitovo to the first holding, km. During this time, the vehicle moves without load, km; L_{Bi} – distance between two neighboring holdings from which it is loaded, km; L_{P_B1} - distance from the last holding from which it is loaded to Plodovitovo, km.

To the total delivery time is added the sum of the downtime and loading of the production in the individual farms:

$$T_{TM} = T_{P\_B1} + \sum_{i=1}^{n} T_{Bi} + \sum_{j=1}^{m} T_{Tj} + T_{P\_B2} \quad (2)$$

where: T_{P_B1} is the travel time from Plodovitovo to the first farm, min; T_{Bi} – duration of movement between two neighboring holdings from which the load is taken, min; T_{Tj} – duration of stay for processing documents and loading at the j-th farm, min; T_{P_B1} – travel time from the last farm from which it is loaded to Plodovitovo, min.

According to Vezirov & Kozlev (2002 and 2006) the carrying capacity coefficient of the vehicle is determined by the dependence:

$$K_T = min\{1, max[V_1,\ V_2, \dots\ V_i\,, \dots] * \rho * \psi\} \quad (3)$$

where: V_1, V_2, … V_i, … are the capacity volumes without or with different superstructures.

With a known load density ρ (kg/m3) and a volume utilization factor ψ, choose the one from the above capacities for which K_t ~1.

Of course, according to the law on road traffic - art. 126 "When transporting goods, the mass of the loaded road vehicle must not exceed the permissible maximum mass reflected in its registration certificate" (Vezirov Ch., Kozlev R. 2002; Vezirov Ch., Kozlev R. 2006; https://lex.bg …; https://rta.government.bg …; https://legislation.apis.bg …; https://iacp- …; Zahariev, I. 2024). For most vehicles, it is less when driving outside the permanent road surface network.

According to Zahariev (2023 and 2024) the volume of transport activities Q, t.km, for each operation is determined by the dependence:

$$Q = L.M, \qquad (4)$$

where: L – transport distance, km; M – mass of transported material, t.

The mass of one salad m_z, kg is determined by measuring 200 pieces of salad (taken at random from the observed arrays) with an electronic scale with a range of 5 kg ±1g. The resulting values are added together. The sum is divided by 200.

RESULTS AND DISCUSSION

Settlements up to 25 km from the village of Plodovitovo

The settlements within a radius of 25 km from the village of Plodovitovo have been determined. The data are placed in Table 1.

Table 1. Distances between the village of Plodovitovo and settlements (https://www.bgmaps.com/map/ …)

Village	The shortest distance between the settlement and the village of Plodovitovo, km	Time to cover the distance, min
Gradina	3.5	5
Orozovo	5.7	7
Krushevo	5.7	8
Mirovo	6.3	7
Parvomay	8.6	11
Opalchenets	9.8	12
Chirpan	10.2	11
Milevo	10.3	10
Cherna gora	11.1	14
Granit	11.6	14
Dobri dol	12.5	18
Vinica	14.1	16
Popovitsa	14.5	14
Turkmen	14.7	18
Karadjalovo	16.8	18
Selci	16.9	17
Zetyovo	17.2	18
Rupkite	17.4	25
Spasovo	17.8	20
Tsenovo	18	21
Ahmatovo	18.1	19
Gorno Belevo	18.2	23
Tatarevo	18.3	24
Partizanin	18.8	17
Boyalino	18.5	23
Byala Reka	19	21
Volovarovo	19.3	20
Bogdanitsa	19.3	20
Belozem	19.5 (22.1)*	(24) 17
Choba	19.6	24
Poroina	19.7	25
Skobelevo	21.3	30
Svoboda	21.3	23
Pravoslaven	21.6	25
Bratya Daskalovi	22.2	27
Dalbok Izvor	22.2	28
Izvorovo	22.4	26
Cheshnigirovo	22.9	20
Darjava	23.1	28
Sredno Gradishte	23.6	27
Brezovo	23.8	29
Konush	24.7	28

* when driving on the A1 motorway.

According to the data in Table 2, there are 42 settlements within a radius of 25 km from the village of Plodovitovo. It is noteworthy that the time to cover 1 km is different for individual sections. This is due to a difference in the geometric characteristics of the road sections and the degree of wear of their coating.

Salad makers

The collected data for small farmers of lettuce from all 42 settlements that work with the exchange are placed in Table 2.

Table 2. Small farmers of lettuce

Village and farmer number	Area planted with lettuce, da	Yield from the area, kg
Gradina 1	0.9	1524.87
Gradina 2	1.1	1863.73
Orizovo 1	2	3388.6
Orizovo 2	0.5	847.15
Orizovo 3	1.6	2710.88
Krushevo 1	1.2	2033.16
Krushevo 2	1.3	2202.59
Krushevo 3	0.65	1101.3
Mirovo 1	0.8	1355.44
Mirovo 2	1.3	2202.59
Parvomay 1	2.5	4235.75
Parvomay 2	5.0	8471.5
Parvomay 3	0.7	1186.01
Opalchenets 1	0.8	1355.44
Chirpan 1	1.4	2372.02
Milevo 1	2.1	3558.03
Milevo 2	0.6	1016.58
Milevo 3	1.8	3049.74
Gerna Gora 1	0.4	677.72
Gerna Gora 2	1.55	2626.17
Granit 1	3.0	5082.9
Granit 2	2.8	4744.04
Granit 3	4.2	7116.06
Dobri Dol 1	1.15	1948.45
Vinitsa 1	2.6	4405.18
Vinitsa 2	0.9	1524.87
Popovitsa 1	1.4	2372.02
Popovitsa 2	2.3	3896.89
Turkmen 1	4.2	7116.06
Turkmen 2	3.3	5591.19
Turkmen 3	0.8	1355.44
Karadjalovo 1	3.6	6099.48
Selci 1	0.95	1609.59
Zetyovo 1	4.7	7963.21
Zetyovo 2	3.1	5252.33
Zetyovo 3	2.8	4744.04
Rupkite 1	2.6	4405.18
Spasovo 1	1.55	2626.17
Tsenovo 1	2.8	4744.04
Tsenovo 2	2.5	4235.75
Ahmatovo 1	3.6	6099.48
Gorno Belevo	2.35	3981.61
Taтarevo	3.2	5421.76
Taтarevo	1.85	3134.46
Partizanin	2.9	4913.47
Boyalino 1	1.6	2710.88
Boyalino 2	1.8	3049.74
Byala Reka 1	3.4	5760.62
Bolovarovo 1	2.7	4574.61
Bogdanitsa 1	4.1	6946.63
Bogdanitsa 2	1.75	2965.03
Belozem 1	4.5	7624.35
Belozem 2	4.2	7116.06
Choba 1	2.2	3727.46
Choba 2	1.9	3219.17
Choba 3	1.8	3049.74
Poroina 1	2.5	4235.75
Poroina 2	0.9	1524.87
Skobelevo 1	2.6	4405.18
Svoboda 1	2.8	4744.04
Pravoslaven 1	3.1	5252.33
Bratya Daskalovi 1	4.8	8132
Bratya Daskalovi 2	4.6	7793.78
Bratya Daskalovi 3	2.3	3896.89
Dalbok Izvor	1.8	3049.74
Izvorovo 1	2.4	4066.32
Cheshnigirovo	2.7	4574.61
Darjava 1	1.85	3134.46
Sredno Gradishte 1	2.3	3896.89
Brezovo 1	4.4	7454.92
Brezovo 2	3.8	6438.34
Brezovo 3	2.6	4405.18
Konush 1	2.0	3388.6

In Table 2, the agricultural producers from the 42nd settlement, namely Plodovitovo, are not marked. In the period 21 - 30.03.2023, 200 lettuces taken from different manufacturers were measured randomly. The average value of 1 piece of salad is m_z = 684g (0.684 kg). In the seeding scheme shown in fig. 1 the average yield is ***Qcc*** = 1,694.3 kg/da (16,943 kg/ha);

The obtained value is close to the official data published in the Annual Report on the State and Development of Agriculture 2021 of the Ministry of Agriculture, Food and Forestry, namely: for 2021 ***Qcc*** = 17,062 kg/ha (https://www.mzh.government.bg …).

30 packages (empty cartons with the plastic bags inside them) were weighed. The mass of the package is m_{op} = 1.986 kg

The total mass of a full box of salads is m_k = 7.368 kg.

Transport scheme

The transport scheme compiled on the basis of the data placed in table 1 is shown in fig.3:

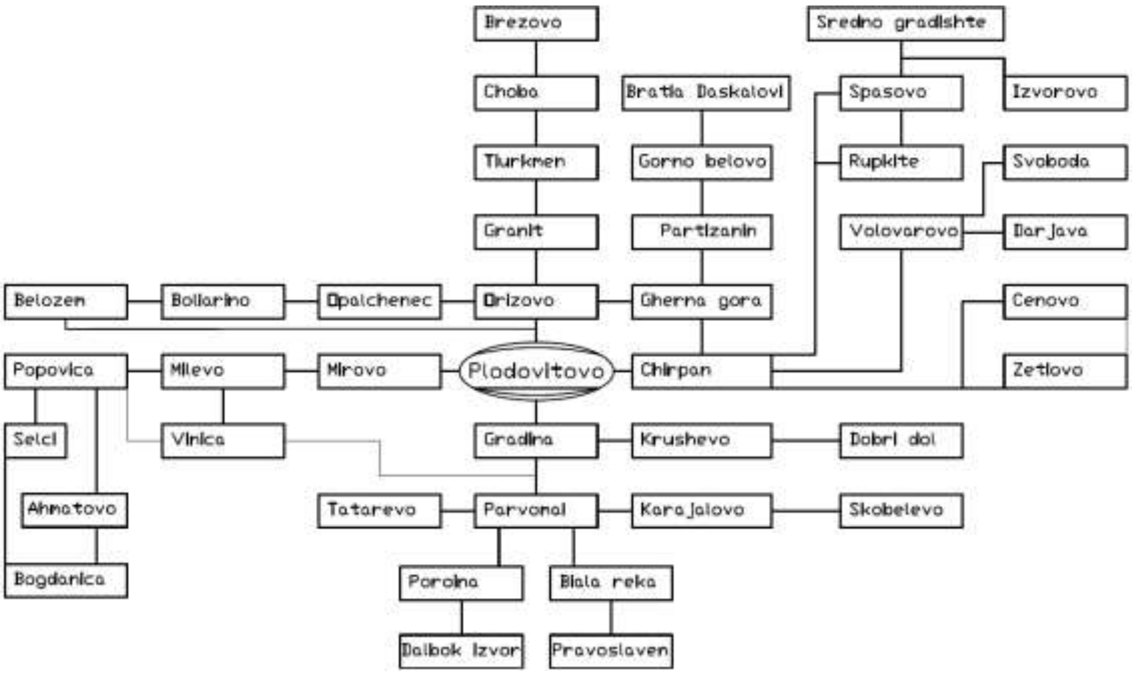


Figure 3. Transport scheme between the village of Plodovitovo and the villages

From fig. 3. the road connections between the village of Plodovitovo and the rest of the settlements in the study are visible. The main road connections are given with thick lines, and the minor ones with thin ones. It is noteworthy that the village of Plodovitovo is located at a crossroads. There are four exits in the four geographical directions.

Road routes and amount of cargo

To determine the parameters of the road routes, the distances between all points on the routes and the amount of cargo needed to be transported during the day were taken into account.

N1. Plodovitovo - Brezovo - Choba - Turkmen - Granit - Orizovo - Plodovitovo (47.6 km);

N2. Plodovitovo - Bratia Daskalovi - Gorno Belovo - Partizanin – Cherna Gora - Orizovo - Plodovitovo (44.4 km);

N3.1. Plodovitovo - Belozem - Bolarino - Oplchenets-Orizovo - Plodovitovo (39 km);

N3.2. Plodovitovo - Belozem - Plodovitovo (44.2 km);

N4.1. Plodovitovo - Sredno Gradishte - Izvorovo - Spasovo - Rupkite - Chirpan - Plodovitovo (53.1 km);

N4.2. Plodovitovo - Sredno Gradishte - Spasovo - Rupkite - Chirpan - Plodovitovo (50.3 km);

N4.3. Plodovitovo - Sredno Gradishte - Spasovo - Chirpan - Plodovitovo (47.2 km);

N4.4. Plodovitovo - Izvorovo - Spasovo - Rupkite - Chirpan - Plodovitovo (47.9 km);

N4.5. Plodovitovo - Izvorovo - Spasovo – Chirpan – Plodovitovo (44.8 km);

N4.6. Plodovitovo - Rupkite - Chirpan - Plodovitovo (34.8 km);

N5.1. Plodovitovo - Cenovo - Chirpan - Plodovitovo (36 km);

N5.2. Plodovitovo - Zetivovo - Chirpan - Plodovitovo (34.4 km);

N5.3. Plodovitovo - Cenovo - Zetovo - Chirpan - Plodovitovo (42.4 km);

N6.1. Plodovitovo - Svoboda - Chirpan - Plodovitovo (42.6 km);

N6.2. Plodovitovo - Darjara - Volovarovo - Chirpan - Plodovitovo (46.2 km);

N6.3. Plodovitovo - Svoboda - Volovarovo - Chirpan - Plodovitovo (48.1 km);

N6.4. Plodovitovo - Svoboda - Volovarovo - Darjjara - Volovarovo - Chirpan - Plodovitovo (56.9 km);

N7. Plodovitovo - Dobri dol – Krushevo – Gradina – Plodovitovo (25 km);

N8. Plodovitovo - Skobelevo – Karadjalovo – Parvomai – Gradina – Plodovitovo (42.6 km);

N9. Plodovitovo – Pravoslaven - Byala Reka - Parvomai - Garden - Plodovitovo (43.2 km);

N10. Plodovitovo - Dalbok Izvor - Poroina - Parvomai - Gradina - Plodovitovo (44.4 km);

N11. Plodovitovo - Tatarevo - Parvomai - Gradina - Plodovitovo (36.6 km);

N12.1. Plodovitovo - Bogdanitsa - Seltsi - Popovitsa - Milevo - Mirovo - Plodovitovo (38.6 km);

N12.2 Plodovitovo - Akhmatovo - Popovitsa – Milevo – Mirovo – Plodovitovo (36.2 km);

N12.3. Plodovitovo – Akhmatovo – Bogdanitsa – Seltsi – Popovitsa – Milevo – Mirovo – Plodovitovo (40.2 km);

N12.4. Plodovitovo - Vinica – Milevo – Mirovo – Plodovitovo (28.2) km;

It is noteworthy that there is only one route (N1, N2, N6, N7, N8, N9, N10 and N11) from the settlements to the vegetable market, with several others possible. In the presence of one route, the possibilities of combining are small. With variant N4, there are 6 sub-variants, which makes it possible to combine.

Transport work, t*km

Based on the routes defined above, the yield from each grower and the harvest period of lettuce ready for sale, the transport work for each route is determined daily. The data are presented in tabular form (Table 3) and shown in figures 4 and 5.

Table 3. Transported cargo and transport work daily for the period of harvesting the ready-to-market salads - 22 days

Transport route	Total cargo transported daily, t	Daily transport work, t*km	Coefficient, t/km
N1	4,13	68,28	0,250
N2	2,44	43,64	0,136
N3.1	1,81	31,57	0,104
N3.2	0,92	20,35	0,042
N4.1	1,08	23,35	0,050
N4.2	0,83	16,09	0,043
N4.3	0,55	10,13	0,030
N4.4	0,84	16,18	0,044
N4.5	0,57	10,17	0,032
N4.6	0,42	6,25	0,028
N5.1	0,71	11,52	0,044
N5.2	1,27	21,66	0,074
N5.3	1,83	35,77	0,094
N6.1	0,44	7,78	0,025
N6.2	0,63	11,29	0,035
N6.3	0,73	14,25	0,037
N6.4	0,93	21,79	0,040
N7	0,67	4,35	0,103
N8	1,73	21	0,143
N9	1,77	20,2	0,155
N10	1,63	21,06	0,126
N11	1,61	19,13	0,135
N12.1	1,81	27,87	0,118
N12.2	1,47	20,06	0,108
N12.3	2,19	37,14	0,129
N12.4	1,06	12,28	0,091

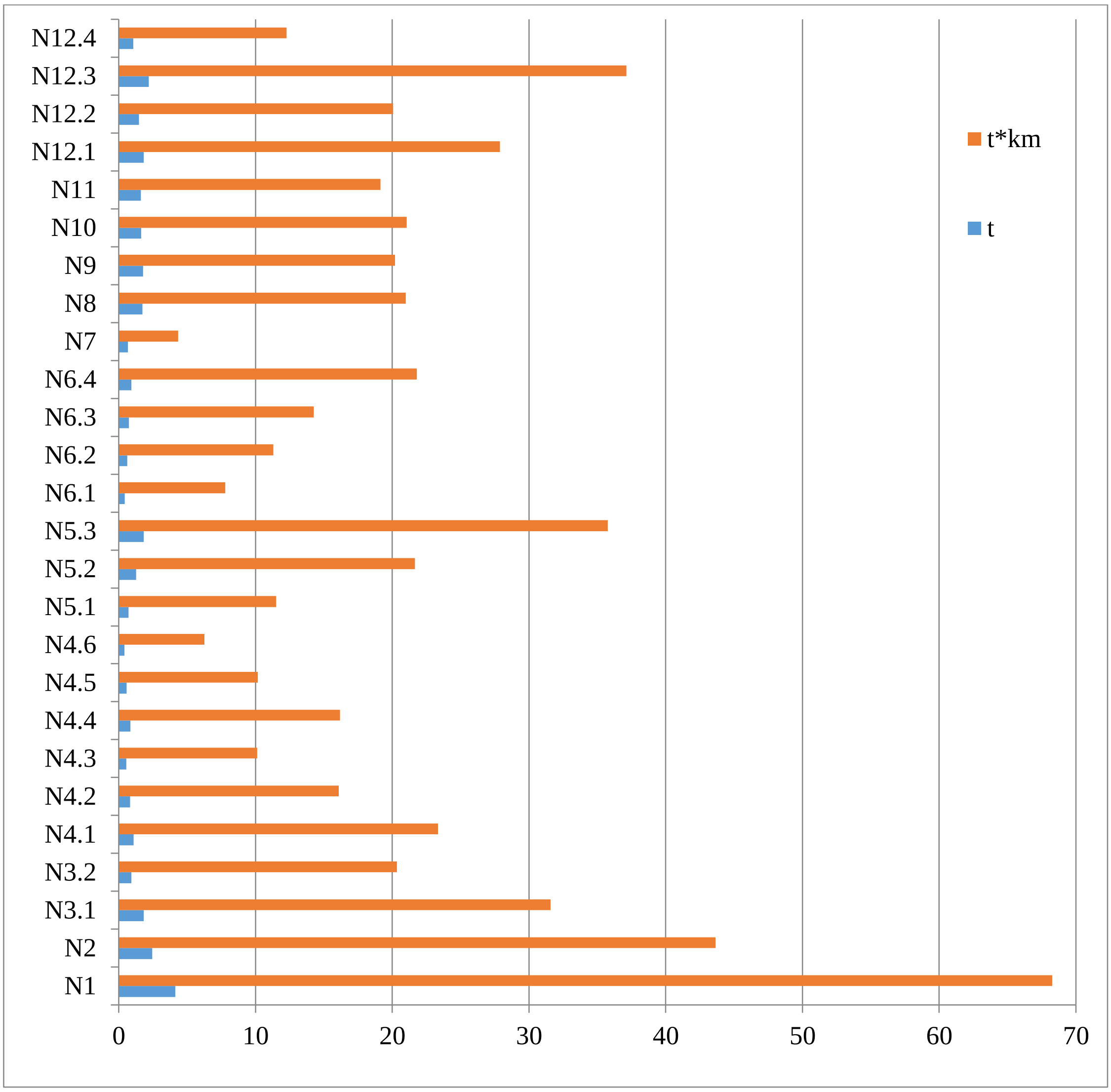


Figure 4. Transport work and the amount of salads transported daily

From the graph in Fig.4. it is evident that only on 3 routes (N1, N2 N12.3) there is a total load over 2t. For the rest, this value is not exceeded, which means that it can be transported by small trucks, for which a license and payment of "toll" fees are not required, but annual vignettes for using the road network outside populated areas.

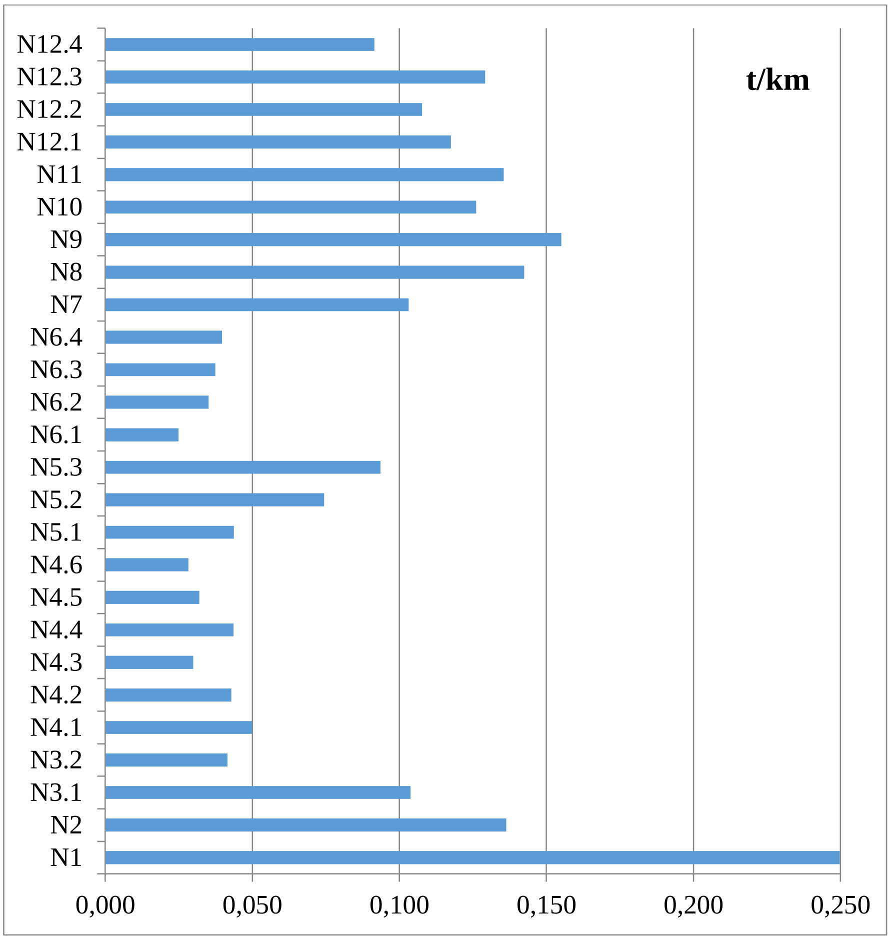


Figure 5. Coefficient φ, t/km

From figure 5 it is clear that the greatest value of the coefficient φ=0.250 is for route N1, followed by N9 φ=0.155, N8 φ=0.143, N2 φ=0.136 and N11 φ=135. The smallest value is reported for route N6.1 φ=0.025 followed by N4.6.

When choosing a route for the movement of a means of transport from several options, it should be taken into account that the coefficient φ has the largest possible value. That is, the largest amount of cargo to be transported over the shortest distance. Another factor that must be taken into account is the carrying capacity factor of the means of transport Kt.

Due to the fact that the cargo is salad, which requires fast delivery from the field to the end user, it is necessary to agree in advance the prepared daily amount of salad from each point. This indicator is also influenced by the market situation during the previous two days. If there is a product in the warehouse, it is not necessary to take the expected average quantity for the day, as the salad can be left in the field and in the following days be prepared for transport, depending on the agreements. In this way, losses from scrapped or revalued goods - salad will be reduced.

CONCLUSIONS

On the basis of the above, can be formulating the following conclusions:
1. The settlements within a radius of 25 km from the village where the vegetable market is located have been determined - 42;
2. The small agricultural producers of lettuce in each of 42 villages and their areas planted with lettuce were determined;

3. The average value of one salad and the yield in the used planting scheme were determined;
4. The transport scheme between the 42 villages and the vegetable market has been drawn up, and the routes for the movement of means of transport have been determined. Some routes have sub-variants, while others do not.
5. The transport work for each individual route is determined;
6. Due to the fact that lettuce quickly withers and loses its freshness, a recommendation has been made for a daily advance agreement to prepare different quantities depending on the availability in the warehouse and the market situation, which will lead to a reduction in losses from scrapped or revalued (reduced) salads.

REFERENCES

Llorach, R., A. Martínez-Sánchez, F.A. Tomás-Barberán, M.I. Gil, F. Ferreres. 2008. Characterisation of polyphenols and antioxidant properties of five lettuce varieties and escarole, Food Chemistry, 108(3): 1028-1038, https://doi.org/10.1016/j.foodchem.2007.11.032

Pereira, E.L. A. Rodrigues, E. Ramalhosa.2013. Influence of working conditions and practices on fresh-cut lettuce salads quality, Food Control, Volume 33, Issue 2, 2013, Pages 406-412, ISSN 0956-7135, https://doi.org/10.1016/j.foodcont.2013.03.021;

https://agriculture.ec.europa.eu/common-agricultural-policy/income-support/additional-schemes/payments-small-farmers_bg 2024/06/17;

Barbov S., D. Lambev. 1976. Organization and management of railway transport in Bulgaria. S., Technology;

Breshkov, I. 1993. Transport Management. S., ed. Farming;

Vasilev, V. 2004. Management of transport systems. S., Economy, p. 39;

EU Directives on Transport Policy, by Mode of Transport, Road Design Rules 1987 (1987);

Tsankov, S. 1994. The role of transport in increasing public production. S., Economy;

Zahariev, I. 2023. Determination the volume of transport operations when harvesting wine grapes from small areas, IOSR Journal of Engineering (IOSRJEN), 13(12): 2278 -8719, -I, PP 67-74;

Radkov, J. 2005. Strategic priorities for the development of transport during economic growth and integration in the unified transport system of Europe, Economic Studies, 19: 2;

https://eurocode.bg/index.php, 2023/09/21;

https://www.gli.government.bg/sites/default/files/upload/documents/2020-09/NAREDBA_16_ot_31051999_g_za_fiziologicni_normi_i_pravila_za_rycna_rabota_s_tejesti.pdf, 2023/09/21;

Zahariev, I. 2024. Determining the time for harvesting grapes from small areas in the Karlovska rose valley, IOSR Journal of Engineering, 14(1): 2278-8719, I, PP 24-31;

https://www.viamichelin.com/ 2024/05/23;

https://titul.bg/10972890445246415856/ 2024/05/23;

https://iisda.government.bg/ras/executive_power/townhall/5981 2024/05/23;

Vezirov Ch., Kozlev R. 2002. Technological service in agriculture, Part I - Transport service, Ruse;

Vezirov Ch., Kozlev R. 2006. Technological service in agriculture, Ruse;

https://lex.bg/laws/ldoc/2134649345, 2024/06/17;

https://rta.government.bg/upload/9167/zdvp.pdf, 2024/06/17;

https://legislation.apis.bg/doc/944/0, 2024/06/17;

https://iacp-sofia.mvr.bg/ruse/, 2024/06/17;

Zahariev, I. 2024. Determination of the volume of transport operations when transporting white cabbage variety "Dabene 5" from the plantation to the regional vegetable market,

International Journal of Advances in Engineering and Management (IJAEM), 6 (03):406-417 www.ijaem.net ISSN: 2395-5252;
https://www.bgmaps.com/map/ … 2024/06/17;
https://www.mzh.government.bg/media/filer_public/2021/12/07/ad_2021.pdf, 2024/06/17.

VEGETATIVE BEHAVIOR OF CABBAGE GROWN WITH ORGANIC FERTILIZATION

Kostadin KOSTADINOV[1], Stoyan FILIPOV[1], Nadezhda SHOPOVA[2], Radoslav CHIPILSKI[3]

[1]Agricultural University – Plovdiv, AU, 12 Mendeleev Blvd, 4000 Plovdiv, Bulgaria
[2]Climate, Atmosphere and Water Research Institute at Bulgarian Academy of Sciences (CAWRI-BAS), 66, Blvd Tzarigradsko chaussee, 1784 Sofia, Bulgaria
[3]Institute of Plant Genetic Resources, 2 Drouzhba Str., 4122 Sadovo, Plovdiv district, Bulgaria, Agricultural Academy, Sofia, Bulgaria

Corresponding author email: kostadinov8888@gmail.com

ABSTRACT

Although mineral fertilizers are essential for vegetable production, their use can have negative consequences both on the quality of production and on the health of the soil and the environment. Therefore, organic fertilization is a good alternative to mineral fertilization and attracts a wide range of research related to food quality. This publication investigates the influence of mineral and organic fertilization on the growth and productivity of cabbage under abiotic stress conditions during the hot summer months. A three-year experiment with mid-early head cabbage variety "Ergensko" used different fertilization was introduce into practice in the field of the Agricultural University of Plovdiv. In the harvestable maturity phase, important productive indicators were evaluated. It has been established that plants grown under organic fertilization conditions have a significant biological potential, with their biometric indicators approaching those of mineral fertilizers.The variant fertilized with the organic fertilizer (Vitaorganic) was determined to have the highest sensory assessment and most preferred taste qualities. The study looks upon to Vitaorganic fertilizer as promising in growing mid-early cabbage with planting in June.

Key words: Cabbage, organic fertilization, productivity, vegetative behavior.

INTRODUCTION

In spite of the use of innovative agricultural techniques and methods, future yields can still be influenced by unfavorable climatic conditions. The size of the harvested area, planted with cabbage in Europe, reached its peak in 2012. Then, in the period 2013 – 2022 this area size had a lower value (Europe's Cabbage Market Report 2024). It is important to note that the rate of its growth in Bulgaria was fastest in 2016, when the harvested area increased compared to the previous year. From 2017 to 2022 the rate of its increase in our country remained a bit lower. To meet the demand for this product, it is important for the agricultural producers to optimize the cultivation technology, including optimal planting times, plant fertilization and irrigation standards, as well as choice of varieties and hybrids (Lapasov et al., 2017; Shokirov et al., 2021). Cabbage (*Brassica oleracea L.*) is a member of the family of cabbage crops and an important fresh and processed vegetable in most countries of the world. Cole crops are biennials, but are generally grown as annuals. They are suitable for the climate of many regions (Chiang et al., 1993; Hasan and Solaiman, 2012). They are known for their nutritional benefits because they are high in carotenoids, vitamins A, B_1, B_2 and C (Drozdowska et al., 2020), calcium, iron, magnesium, and dietary fiber (Guerena, 2006). Cole crops also contribute a substantial amount of protein to the diet (Traka & Mithen 2009; Colon et al, 2016). Cabbage has high requirements for all nutrients, especially nitrogen, and cabbage demands for achieving high yields ranged from 13 to 31 kg N/da (Sanderson and Ivany, 1999; Lešić et. al. 2004; Dumicic et al, 2014). Cabbage accumulates a large amount of organic matter in a short period. It is particularly demanding toward nitrogen and potassium, and also to phosphorus and calcium. It belongs to the group of plants, extracting the most nutrients from the soil. At the moment of harvesting, 39-44% of nitrogen, 42-48% of phosphorus and 38-51% of potassium are drawn from the soil. Nitrogen contributes to yield increase but fertilization only with nitrogen should not be allowed, as the deficiency of phosphorus and potassium worsen the quality and longevity of the cabbage produce (Kostadinov and Borisov 2017). Although mineral fertilization is essential for crop production, the excessive use of mineral fertilizers has negative consequences not only on yield quality but also on agroecosystems (Dumicic et al, 2014; Ahmed el-Henawy et al, 2018). In addition, overdosing of mineral fertilizers is a type of energy loss and can affect the nutrients in the soil (Kaushik and Djiwanti 2017). Nitrogen overuse in modern agriculture is of major importance for the quality of plant products (Dumicic et al, 2014). Therefore, organic fertilization is a good alternative to mineral fertilization and attracts a wide spectrum of research activities, related to studying the nutritional value, quantity and quality of foods. Organic fertilization increases soil biological activity, improves nutrient mobilization and soil structure, and increases soil water retention (Dumicic et al, 2014). Several studies have illustrated the positive effect of the combined use of mineral and organic fertilizers in fields, which for years in a row have continuously received only N, P and K, without any trace elements or organic fertilizers (Kaur et al. 2005; Chand et al. 2006). Last, but not least, the cultivation of field vegetable crops, including cabbage, is directly related to climate change and the trend for air temperature increase in the studied region (Slavcheva et al. 2020), and to the change of the main agrometeorological conditions in the country (Georgieva et al., 2022). The aim of the present study is to determine the effect of organic fertilization on the vegetative behavior, yields and quality of cabbage plants under conditions of abiotic stress during the hot summer months in three consecutive years.

MATERIALS AND METHODS

The experimental work was conducted in the period 2021-2023 in the Experimental Base of the Department of Vegetable Farming at the Faculty of Viticulture and Horticulture of the Agricultural University of Plovdiv with mid-early head cabbage variety "Ergensko" and planting date at the beginning of June.

Meteorological data. The meteorological conditions were monitored during the studied period by means of an automatic station in the experimental area. Daily meteorological data, including daily maximum and average air temperatures in °C and relative air humidity in percent were measured and processed in the period 2 June – 25 September. The phenological development of the cabbage was monitored in accordance with the international BBCH classifier (Meier, U. (2001), i.e., the plants were planted in the growing stage (4th true leaf unfolded-5th true leaf unfolded) and harvested in the stage of development of the harvestable vegetative parts (80% < typical size, form and firmness of heads reached). The growing technology included a drip irrigation system, meaning that measured rainfall had a lower influence.

Seedling production. Sowing was carried out in a nursery at the beginning of May. Organic mixture for seedlings was used - 80% perlite, 20% lumbricompost, developed for seedling bioproduction (Kostadinov and Filipov, 2013).

Planting. The plants were planted in the phase of growth at the moment of 4-5th leaf formation (4th true leaf unfolded - 5th true leaf unfolded) on the 2nd of June in the open air in a

2-row planting strip, by the scheme 90+70/50cm, with a profile of the soil surface high flat bed. The experiment was conducted by a block method in three variants (1. Control – unfertilized; 2. Mineral fertilization (NPK); 3. Vitaorganic) in four repetitions, with 20 plants for each repetition and size of the experimental area of 10 m^2. Fertilizers were used as main fertilization with preparation of the soil before planting in doses: N- 17.6 kg/da, P_2O_5- 7.2 kg/da, + K2O – 17.6kg/da, Vitaorganic - 100kg/da. The measurements were performed in the growing stage development of harvestable vegetative plant parts about the end of September.

Biometric studies. By means of biometric studies and morphological analyses, the most important sides of the cabbage vegetative behavior in the growing stage development of harvestable vegetative plant parts (80% < typical size, form and firmness of heads reached) has been tracked for 16 plants per variant for each variety. The assessment of the vegetative development of the plants in harvestable maturity (growing stage development of harvestable vegetative plant parts - 80% < typical size, form and firmness of heads reached) was made for 16 plants per variant about the end of September. The studied parameters were: fresh mass of the whole plant (kg); rosette – diameter (cm), mass (kg); number of leaves; stem - diameter (mm), mass (kg); and height (cm).

Production quality. An average sample of 16 well-ripened cabbage heads for each cultivation variant was analyzed by the parameters: dry matter content determined by weight (by drying ground plant material at 500 °C to constant weight); ascorbic acid by Tillman reaction with 2,6-dichlorophenolindophenol (Ghenadiev et al., 1969); total sugars by Schoorl Regenbogen (Gennadiev et al., 1969). A 5-point scale (taste Ranking test) was used, with the lowest score being 1 and the highest score being 5.

RESULTS AND DISCUSSION

Meteorological conditions. Local climate conditions greatly affect cabbage production, primarily plant growth, occurrence and development of diseases, harmful insects, and weeds (Cervenski et al, 2022). These authors report that certain abiotic stresses can be minimized by irrigation and fertilization, whereas others, such as fluctuating air temperatures, are more difficult to overcome. Although adapted to different climatic and soil conditions, cabbage prefers colder and wetter growing regions (Matotan, 2006). It is a cool season crop, which tolerates light frost and even short-term hard freezes, but prolonged deep freezes are fatal for it (Capinera, 2020). The following temperature ranges are pointed out by the different authors as optimum for the head formation phenophase: 17.7 to 20°C (Howe and Waters, 1994), 16 to 20°C (Hara and Sonoda, 1982, Criddle et al., 1997), 15 to 18℃ (Lešić et al., 2004), 17.2 to 19.9°C (Žnidarčič et al., 2007), 14 to 24°C (Toth et al., 2011), 16.1 to 16.8°C (Kołota et al., 2015), 17°C and 22°C (Nurhidayati et al., 2016), 17.5-19.1°C (Paranhos et al., 2016) and 15 to 18°C (Červenski and Medić-Pap, 2018). The temperatures, which can cause heat stress and adversely affect cabbage head formation and yield, according to various sources, are: above 19°C (Everaats, 1990),above 25°C (Matotan, 2006), above 24°C (Kahn et al., 2007) and above 30°C (Červenski and Medić-Pap, 2018). Optimal growth occurs at an average daily temperature above 16°C with an average daily maximum of 24°C and daily amplitude of about 14°C.

The average temperatures in all considered months of the three experimental years significantly exceeded the optimal values of 17°C (Figures 1 and 2).

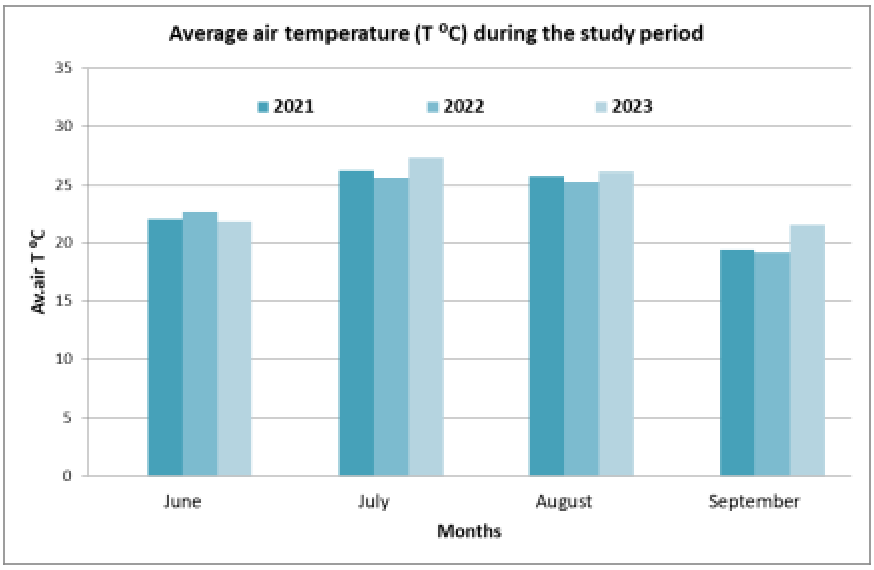


Figure 1. Average daily temperatures AU-Plovdiv

The last of the years – 2023 – was with the lowest average temperature in the initial period of rooting and adaptation of the seedlings in June, which had a favorable effect on the cabbage development in its later phases.

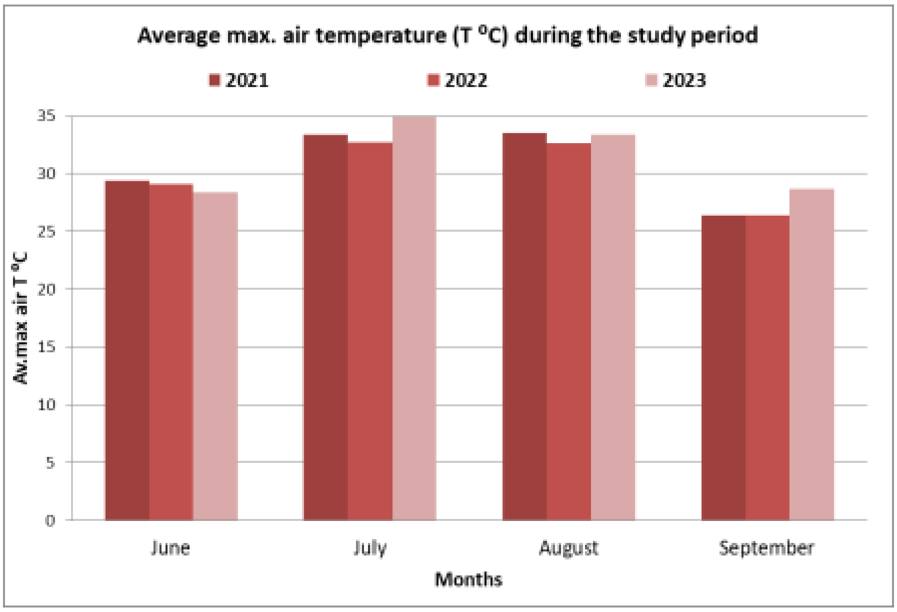


Figure 2. Average of daily maximal temperatures AU-Plovdiv

The highest temperatures in 2023 were seen in July and August, but combined with optimal irrigation, they had no adverse effect on the major morphological parameters. That same year, September was warmer, which favored the late stages of the cabbage development. The average of the maximum values was highest in July 2023, when it reached 35°C.

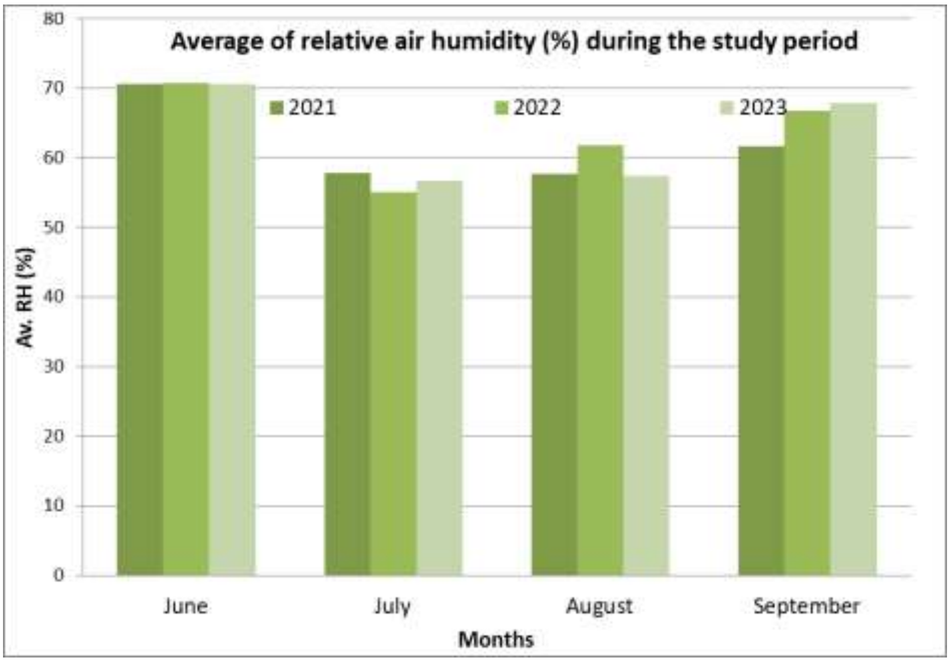


Figure 3. Average of relative air humidity % AU-Plovdiv

Relative air humidity (RH%) is an important meteorological parameter during the period of growth and formation of the product part (cabbage head). The crop requirements for average relative humidity are within 60 to 90 %. The values for the three experimental years were close – about 71% in the initial period (June), as well as in the period of harvestable ripeness – about 80% (Figure 3).

During the warmest months every year, the values were between 62% and 55%. According to FAO the yield under irrigation conditions reaches 2500 kg/da to 3500 kg/da with a maximum of 5000 kg/da in case of spraying and good fertilization. The duration of the growing season of cabbage varies depending on the climate, variety, and planting date, and in the particular case of the experiment it was 115 days. Typical of the species is the slower rate of development in the early phases (the first half of the growing season), which shows up well here even at the measured above-normal temperatures. During the next phase of growth, the plant doubles its weight every 9 days for about 50 days. The period of ripening is 15 days, and the harvest is collected around the 25th of September.

Vegetative parameters

The number of leaves together with the diameter and mass of the leaf rosette determine to a great extent the biological potential of the plant and the amount of its yield. Fertilization and climatic conditions during the growing period strongly influence on the biometric parameters of the leaf rosette. A variance analysis of the change in the factors fertilization and year conditions was made (Table 1). From the obtained average values and in accordance with the three measured parameters (number of leaves, diameter and mass of the leaf rosette), the best growth stands out in the option with conventional fertilization compared to the other variants. The increase in the weight of the leaf rosette is the highest. Dumičić et al., 2014 declared obtaining similar results for the leaf rosette in an experiment with mineral and organic fertilizers. The values of the biological parameters with biological fertilization are also higher than those with the control plants. The statistical analysis of the influence of this factor shows significant differences between the two types of fertilization and the control, on the one hand, and between the conventional and biological fertilization, on the other hand. The meteorological conditions year by year also have a statistically significant influence on the values of the parameters, considered in the study. The strongest influence of this factor has been recorded for the diameter of the leaf rosette, with an increase from the first to the last year seen with the three variants of fertilization. A reason for this result can be the lower air temperatures in June 2023, and the warmer autumn season in the same year. The plants, grown at a cooler initial period and higher temperatures during ripening, have the largest diameter of their leaf rosette. The influence is weaker for the number of leaves and weight of the rosette, and there is no positive gradation in the years, especially regarding the number of leaves per leaf rosette.

Table 1. ANOVA: two factors with replication of the leaf rosette biometric indicators

Control						
year	Diameter, cm		Number of leaves		Weight,kg	
2021	78.4	lsd fertilizer	14.1	lsd fertilizer	1.12	lsd fertilizer
2022	80.3	**5%=5.08**	18.2	**5%=2.05**	0.93	**5%=0.16**
2023	84.2	**1%=6.75**	17.0	**1%=2.72**	1.36	**1%=0.21**
average	**80.97**	**0.1%=8.75**	**16.43**	**0.1%=3.52**	**1.14**	**0,1%=0.27**
Conventional fertilization						
year	Diameter, cm		Number of leaves		Weight, kg	
2021	86.1	lsd year	20.0	lsd year	1.58	lsd year
2022	94.9	**5%=5.08**	24.2	**5%=2.05**	1.69	**5%=0.16**
2023	98.9	**1%=6.75**	19.3	**1%=2.72**	1.61	**1%=0.21**
average	**93.30**	**0.1%=8.75**	**21.17**	**0.1%=3.52**	**1.63**	**0.1%=0.27**
Biological fertilization						
year	Diameter, cm		Number of leaves		Weight, kg	
2021	82.8	lsd interaction	17.0	lsd interaction	1.39	lsd interaction
2022	92.8	**5%=8.81**	21.4	**5%=3.54**	1.30	**5%=0.27**
2023	95.6	**1%=11.69**	17.4	**1%=4.70**	1.50	**1%=0.36**
average	**90,40**	**0,1%=15.16**	**18.60**	**0.1%=6.10**	**1.40**	**0.1%=0.46**

The measured biometric indicators height of the whole plant, diameter of the leaf rosette, weight and diameter of the cabbage stem and average mass of the whole plant, are presented in Tables 2 and 3.

Obtaining similar results was reported by Haque et al., 2022 in a comparative study with organic and NPK fertilizers for cabbage plants. The variant with biological fertilization is also with higher values compared to the unfertilized option. The calculated variance for this factor shows statistically significant differences for all taken parameters between the conventional fertilization and the control, on the one hand, and between the conventional and biological fertilization, on the other hand, except for the stem dimeter indicator in biological fertilization. Similar to the results, concerning the leaf rosette, the biological fertilization is statistically significantly different, compared to the control. Ahmed el-Henawy et al. 2018 concluded in a study of red cabbage with nano-Se, nano-Cu, compost tea and NPK fertilizers that the vegetative parameters were higher compared to the unfertilized control plants. The meteorological conditions by year have a strong and statistically significant influence on the values of the cabbage weight and height and the average mass of the whole plant. As with the leaf rosette diameter, the weight goes up, reaching its peak in 2023. For the parameters cob diameter and diameter of the entire plant, the influence is weak, and, in most cases, statistically not significant. The analysis of the results shows the strongest growth in the leaf rosette and the highest average mass of the whole plant in the conventionally fertilized plants (Table 3).

Table 2. ANOVA of the two factors on the cabbage head biometric indicators

Control						
year	Height of cabbage head, cm		Diameter of cabbage head, cm		Diameter of stem of cabbage head, mm	
2021	15.83	lsd fertilizer	18.67	lsd fertilizer	34.92	lsd fertilizer
2022	15.06	**5%=0.89**	18.76	**5%=0.82**	36.73	**5%=3.38**
2023	18.89	**1%=1.18**	20.67	**1%=1.09**	34.38	**1%=4.49**
average	**16.59**	**0.1%=1.53**	**19.37**	**0.1%=1.42**	**35.34**	**0.1%=5.82**
Conventional fertilization						
year	Height of cabbage head, cm		Diameter of cabbage head, cm		Diameter of stem of cabbage head, mm	
2021	17.44	lsd year	20.61	lsd year	44.69	lsd year
2022	18.94	**5%=0.89**	22.11	**5%=0.82**	44.05	**5%=3.38**
2023	19.89	**1%=1.18**	23.00	**1%=1.09**	45.30	**1%=4.49**
средно	**18.76**	**0.1%=1.53**	**21.91**	**0.1%=1.42**	**44.68**	**0.1%=5.82**
Biological fertilization						
year	Height of cabbage head, cm		Diameter of cabbage head, cm		Diameter of stem of cabbage head, mm	
2021	17.17	lsd interaction	19.67	lsd interaction	42.85	lsd interaction
2022	16.44	**5%=1.54**	20.28	**5%=1.43**	41.06	**5%=5.86**
2023	19.11	**1%=2.05**	21.33	**1%=1.89**	42.71	**1%=7.78**
average	**17.57**	**0.1%=2.66**	**20.43**	**0.1%=2.46**	**42.21**	**0.1%=10.09**

Table 3. ANOVA of the two factors on the cabbage head and whole plant biometric indicators

Control				
year	Weight of cabbage head, kg		Average mass of the whole plant, kg	
2021	2.16	lsd fertilizer	3.28	lsd fertilizer
2022	2.37	**5%=0.16**	3.30	**5%=0.49**
2023	2.51	**1%=0.22**	3.87	**1%=0.66**
average	**2.35**	**0.1%=0.28**	**3.48**	**0.1%=0.85**
Conventional fertilization				
year	Weight of cabbage head, kg		Average mass of the whole plant, kg	
2021	2.74	lsd year	4.32	lsd year
2022	3.34	**5%=0.16**	5.03	**5%=0.49**
2023	3.68	**1%=0.22**	5.29	**1%=0.66**
average	**3.25**	**0.1%=0.28**	**4.88**	**0.1%=0.85**
Biological fertilization				
year	Weight of cabbage head, kg		Average mass of the whole plant, kg	
2021	2.41	lsd interaction	3.80	lsd interaction
2022	2.70	**5%=0.28**	4.00	**5%=0.86**
2023	3.32	**1%=0.38**	4.82	**1%=1.13**
average	**2.81**	**0.1%=0.49**	**4.21**	**0.1%=1.47**

Cabbage productive behavior in the period 2021-2023. From the two-factorial variance analysis, performed for determining the yield variation in accordance with the factors meteorological conditions/year and type of fertilization, F values, higher than the critical F values were registered, the same being true also for the interaction (Tables 4 and 5).

Table 4. ANOVA of the cabbage productivity

Source of Variation	SS	df	MS	F	P-value	F crit	μ influence
Type of fertilization	13382568.04	2	6691284	192.25	7.15E-13	3.55	37.90
years	20073059.28	2	10036530	288.36	2.13E-14	3.55	56.85
Interaction	1224759.147	4	306189.8	8.80	0.000405	2.93	3.47
Error	626490.5548	18	34805.03				
Total	35306877.02	26					

This indicates a very good confidence at the 0.1%/p<0.001 level for the difference between the yield values under the influence of the two factors and the interaction. The calculated percentage of the total variation for the influence of each factor individually and the interaction between them, shows that the strongest influence on the yield has the factor year conditions, while the interaction between the factors is with the weakest effect. We can conclude from these results that both factors (the meteorological conditions and the applied types of fertilization), considered independently of each other, have affected the cabbage variety yield (Tables 4 and 5) in the studied period.

Table 5. Cabbage yield when applying different types of fertilization over a three-year period

Variant of fertilization	**Repetitions**	**Yield, kg/da**			**Average fertilizing variants**
		2021	**2022**	**2023**	
1 control	I	5566.1	6106.01	7120.3	6264.1
	II	5850.97	6418.51	7484.72	6584.7
	III	5674.3	6224.71	7258.72	6385.9
	Average values 2021	5697.1	6249.7	7287.9	**6411.6**
2 NPK	I	6711.979	8181.9	9014.19	7969.4
	II	6986.966	8517.11	9383.5	8295.9
	III	6849.246	8349.23	9198.54	8132.3
	Average values 2022	6849.4	8349.4	9198.7	**8132.5**
3 Vitaorganic	I	6153.583	6892.02	8473.49	7173.0
	II	6530.333	7313.98	8992.28	7612.2
	III	6279.103	7032.6	8646.33	7319.3
	Average values 2023	6321.0	7079.5	8704.0	**7368.2**
	Average values by year 2021-2023	**6289.2**	**7226.2**	**8396.9**	
LSD 5% Fertilization Type	184.8	lsd 5% year	184.8	lsd 5% interaction	320.0
lsd 1% Fertilization Type	253.2	lsd 1% year	253.2	lsd 1% interaction	438.4
lsd 0.1% Fertilization Type	344.9	lsd 0.1% year	344.9	lsd 0.1% interaction	597.3

The continuation of the analysis with the calculated least significant differences of the average yield values is given in Table 5. It becomes clear from the results that the average value of the yield for all types of fertilization and the control is the highest and significant to the level of 0.1% during the growing season of 2023. From the obtained significance of the differences, we can also conclude that the meteorological conditions for each individual year have affected the yield differently and significantly up to 0.1%. The variance of the typical yield with the type of the fertilization factor is in a similar trend, with the average yield value for a three-year period for the variant with mineral fertilization being the highest and significant up to 0.1% compared to the other options. The yield in the biological fertilization variant, on the other hand, is higher than the control, and significantly different not only from the control but also from the case with mineral fertilization. The biologically fertilized plants outperform the control and are similar in their productive performance, compared to the conventionally grown plants in all three experimental years for the three planting dates.

Impact of organic fertilization on the quality of production 2021-2023. Analyses showed that organic production systems, which omit the use of agrochemicals, produce crops with lower yields, but superior nutritional composition (Rempelos et al., 2023; Gelaye, 2024). The same studies confirm that higher concentrations of phenolics and other nutritionally desirable phytochemicals are found in organic vegetables. In this study, the fertilization options were compared in terms of Dry matter (%), Ascorbic acid (mg %), Total sugars (%) and a sensory assessment was made (Table 6). The content of dry matter in the unfertilized plants is the lowest – 8.92%. After biological fertilization, it increases by 4.04% compared to the control, and approaches the level, contained in the plants, fertilized with NPK (Table 6).

Table 6. Average value of chemical components and sensory assessment on a 5-point scale

Variant	Dry matter (%)	Ascorbic acid (mg %)	Total sugars (%)	Sensory assessment (1-5)
1. Control (unfertilized)	8.92	36.84	4.06	4.5
2. NPK (MT)	10.15	38.75	4.68	4.1
3. Vitaorganic	9.28	31.11	4.18	4.8

The biologically fertilized plants with Vitaorganic have a 2.96% higher content of sugars compared to the control plants. The concentration of the total carbohydrates is comparable to the option with mineral fertilization. The lowest value – 4.06% - is the value, measured in the control variant. The sensory assessment shows that the cabbage plants, grown after biological fertilization with Vitaorganic, have the most preferred taste quality, and the value of this indicator is close to the maximum 4.8. The plants, grown without fertilization, are next in order with 4.5. The lowest is the assessment of the plants, fertilized with mineral fertilizers – (4).

CONCLUSIONS

This study considers the influence of different variants of fertilization on the biological behavior of cabbage within a period of three consecutive years – 2021-2023. The main chemical components in the harvested cabbage produce and its taste quality have been assessed for plants, fed with mineral (NPK) and organic fertilizers (Vitaorganic).

1. Organically fertilized plants (Vitaorganic) have a substantial biological potential and productive parameters, close to those, of plants, grown with mineral fertilization (NPK),

under different climatic conditions. Both fertilization variants show statistically significant differences with respect to the control.

2. The meteorological conditions for each individual year affect the yield differently and significantly up to 0.1%.
3. Organic fertilization leads to accumulation of amounts of dry matter and total sugars, similar to the ones, accumulated in plants, grown with mineral fertilization.
4. The sensory assessment shows that the most preferred taste qualities have the leaf rosette, grown after biological fertilization with Vitaorganic, followed by the unfertilized control plants.
5. The conducted study gives grounds to recommend the biological fertilizer Vitaorganic as a promising option for growing mid-early cabbage with planting in June.

ACKNOWLEDGEMENTS

The research was carried out with the funding of the Center for Scientific Research, Intellectual Property Protection and Technology Transfer at the Agricultural University - Plovdiv.

REFERENCES

Capinera J. (2020). Handbook of Vegetable Pests (Second Edition), Elsevier Inc. All

Chiang M., Chong C, Landry B., and R. Crete. (1993). Cabbage: *Brassica oleracea subsp. capitata L* in Genetic Improvement of Vegetable Crops, pp. 113–155, Elsevier, Amsterdam,Netherlands.

Červenski J. Vlajić S., Ignjatov M., Tamindžić G., & Zec S. (2022). Agroclimatic conditions for cabbage production. Ratarstvo i povrtarstvo, 59(2), 43-50. https://doi.org/10.5937/ratpov59-3677

Červenski, J., & Medić-Pap, S. (2018). Proizvodnja kupusa, Cabbage Production. Institute of Field and Vegetable Crops, NoviSad

Chand, S., Anwar, M. & Patra, D.D. (2006). Influence of long-term application of organic and inorganic fertilizer to build up soil fertility and nutrient uptake in mint-mustard cropping sequence. Communications in Soil Science and Plant Analysis, 37, 63-76.

Colon, B., Toor. (2016). Chapter Three - A Review of Uptake and Translocation of Pharmaceuticals and Personal Care Products by Food Crops Irrigated with Treated Wastewater, Editor(s): Donald L. Sparks, Advances in Agronomy, Academic Press, Volume 140, 2016, Pages 75-100, https://doi.org/10.1016/bs.agron.2016.07.001.

Criddle, R. S., Smith, B. N., & Hansen, L. D. (1997). A respiration-based description of plant growth rate responses totemperature. Planta, 201(4), 441-445. https://doi.org/10.1007/s004250050087.

Drozdowska, M., Leszczy´nska,T.,Koronowicz A., Piasna- Słupecka, E., Domagała D., and B. Kusznierewicz, (2020). Young shoots of red cabbage are a better source of selected nutrients and glucosinolates in comparison to the vegetable at full maturity, European Food Research and Technology, vol. 246, no. 12, pp. 2505–2515, 2020.

Dumicic, G., Vukobratović, M. Vukobratović, Ž. Urlić, B. Žanko, M. & Kudić, H. (2014). Effect of Fertilization on Cabbage Yield Characteristics. The Journal of Ege University Faculty of Agriculture, 2, 399-404.

El-Henawy, A., El-Sheikh, I. Hassan, A. Madein, A. El-Sheikh, A. El-Yamany, A. Radwan, A. Mohamed, F. Khamees, M. Ramadan, M. Abdelhamid, M. Khaled, H. El-Faramawy, H.

Ayoub, Y. Youssef, S. & Faizy, S. E. (2018). Response of Cultivated Broccoli and Red Cabbage Crops to Mineral, Organic and Nano-fertilizers. Environment, Biodiversity and Soil Security, 2, 221-231. doi: 10.21608/jenvbs.2019.6797.1046

Europe's Cabbage Market Report 2024 - Prices, Size, Forecast, and Companies (indexbox.io),

Everaarts, A. P. (1990). Growth, development and yield ofwhite cabbage in relation to time of planting. ActaHorticulturae, 267, 279-288. https://doi.org/10.17660/ActaHortic.1990.267.35

Filipov St., K. Kostadinov, 2013. Influence of the amount of lumbricompost on the quality of the dense seedlings of greenhouse tomatoes. International Scientific Online Journal "Science and Technology", Ed. "Union of Scientists - St. Zagora", Volume III; number 6; 2013: Plant studies. ISNN No. 1314-4111.

http://journal.sustz.com/VolumeIII/Number6/index.htmlGenadiev, A., Kalcheva, D., Nenchev, N., Tevekeliev, N., & Chavdarova, N. (1969). Food analyses. Technique, Sofia (Bg).

Gelaye Y. (2024). A Systematic Review on Effects of Nitrogen Fertilizer Levels on Cabbage (*Brassica oleracea var. capitata L.*) Production in Ethiopia, The Scientific World Journal, vol. 2024, Article ID 6086730, 11 pages, 2024. https://doi.org/10.1155/2024/6086730

Georgieva, V. Kazandjiev, V. Bozhanova, V. Mihova, G. Ivanova, D. Todorovska, E. Uhr, Z. Ilchovska, M. Sotirov, D. & Malasheva, P. (2022). Climatic Changes—A Challenge for the Bulgarian Farmers. Agriculture, 12, 2090. https://doi.org/10.3390/agriculture12122090

Guerena M. (2006). Cole Crops and other Brassicas: Organic Production. ATTRA-National Sustainable Agriculture Information Service. p. 1-19.

Haque, SMR., Mondal MF, Hassan, MK. Islam, MN. Hoque, MMI. Ahamed, S. & Bir, MSH. (2022). Effects of Organic Manures on Growth and Yield of Cabbage. Journal of Agriculture, Food and Environment, 3(2), 45-49.

Hara, T., & Sonoda, Y. 1982. Cabbage-head development asaffected by nitrogen and temperature. Soil Science and Plant Nutrition, 28(1), 109-117. https://doi.org/10.1080/00380768.1982.10432376.

Hasan, M., & Solaiman, AHM. (2012). Efficacy of organic and organic fertilizer on the growth of Brassica oleracea L. (Cabbage). International Journal of Agriculture and Crop Sciences., 4 (3), 128-138.

Howe, T.K., & Waters, W.E. (1995). Two-year summary of cabbagecultivar yield trials. In: Florida State Horticultural Society.Meeting (USA).

https://www.fao.org/land-water/databases-and-software/crop-information/cabbage/fr/).

Kahn, B.A., Edelson, J., & Damicone, J.P. (2007). Cole cropproduction (Broccoli, Cabbage, and Cauliflower). Oklahoma Cooperative Extension Service.

Kaur, K., Kapoor, K.K. & Gupta, A.P. (2005). Impact of organic manures with and without mineral fertilizers on soil chemical and biological properties under tropical conditions. Journal Plant Nutrition and Soil Science, 168, 117-122.

Kaushik, S., & Djiwanti, S.R. (2017). Nanotechnology for Enhancing Crop Productivity. In: Prasad, R., Kumar, M., Kumar, V. (eds). Nanotechnology. (pp.249-262). Springer, Singapore.

Kołota, E., & Chohura, P. (2015). Control of head size and nutritionalvalue of cabbage by plant population and nitrogenfertilization. Acta Scientiarum Polonorum Hortorum Cultus, 2(14), 75-85.

Kostadinov K, Borisov P. (2017). Organic vegetable production. Technology and planning (tomatoes, cucumbers, watermelons, cabbage), 234 (Bg).

Lapasov SS, Shokirov AJ, & Azimov BJ. (2016). International Journal of Science and Research, 6(11), 147-156.

Lapasov, SS., Shokirov, AJ., & Azimov, BJ. (2017). Selection of White Cabbage Variety Samples Those are Cultivated in Uzbekistan Conditions. International Journal of Science and Research, 1999-2002.

Lešić, R. Borošić, J. Buturac, I. Ćustić-Herak, M. Poljak, M. &Romić, D. (2004). Povrćarstvo. Zrinski, Čakovec.

Matotan, Z. (2006). Tehnologija proizvodnje i sortimentkupusnjača. Glasnik Zaštite Bilja, 29, 4-34.

Meier, U. (2001). Growth stages of mono- and dicotyledonous plants. BBCH Monograph.

Nurhidayati, N., Ali, U. & Murwani, I. (2016). Yield and quality of cabbage (Brassica oleracea L. var. Capitata) underorganic growing media using vermicompost and earthworm Pontoscolex corethrurus inoculation. Agriculture and Agricultural Science Procedia, 11, 5-13. https://doi.org/10.1016/j.aaspro.2016.12.002.

Paranhos, L.G., Barrett, C.E. Zotarelli. L. Darnell, R. Migliaccio, K. & Borisova, T. (2016). Planting date and in-row plant spacing effects on growth and yield of cabbage underplastic mulch. Scientia Horticulturae, 202, 49-56, https://doi.org/10.1016/j.scienta.2016.02.022.

Sanderson, K.R. & Ivany, J.A. (1999). Cole crop yield response to reduced nitrogen rates. Canadian Journal of Plant Science, 79, 149-151.

Shokirov AJ, Lapasov S, and Shokirov KJ. (2021). Scientific and practical fundamentals of growing cabbage (Brassica capitata lizg.) in Uzbekistan. IOP Conf. Ser.: Earth Environ. Sci. 939 012044

Slavcheva-Sirakova, D., Shopova, N. Kostsdinov, K. Filipov, S. & Velichkova, K. (2020). Climate Analysis and Effects Of Abiotic Stresson Salad Grounded In Underground Greenhouse and Outdoor And Effects Of Organic Fertilizersin The Fight With Stress Factors. Scientific Papers. Series B, Horticulture. Vol. Lxiv, No. 2.

Rempelos, L.; Bara ´nski, M.; Sufar, E.K.; Gilroy, J.; Shotton, P.;Leifert, H.; ´Srednicka-Tober, D.; Hasanaliyeva, G.; Rosa, E.A.S.;Hajslova, J.; et al. (2023). Effect of Climatic Conditions, and Agronomic Practices Used in Organic and Conventional Crop Production on Yield and Nutritional Composition Parameters in Potato, Cabbage, Lettuce and Onion; Results from the Long-Term NFSC-Trials. Agronomy, 13, 1225. https://doi.org/10.3390/ agronomy13051225

Traka, M and R. Mithen, "Glucosinolates, isothiocyanates and human health. (2009). Phytochemistry Reviews, vol. 8, no. 1, pp. 269–282, 2009.

Toth, N., Fabek, S. Benko, B. Žutić, I. Stubljar, S. & Bertoša, J. (2011). Utjecaj volumena supstrata i starosti presadnica na prinoskupusa. Glasnik Zaštite Bilja, 34(6), 30-38.

Žnidarčič, D., Kacjan-Maršić, N. Osvald, J. Požrl, T. & Trdan, S. (2007). Yield and quality of early cabbage (Brassicaoleracea var. capitata L.) in response to within-row plantspacing, Acta Agriculturae Slovenica, 89 (1), 15–23. https://doi.org/10.2478/v10014-007-0002-1.

ESTIMATING FIELD CAPACITY BY USING MULTIPLE LINEAR REGRESSIONS

Coşkun GÜLSER

Ondokuz Mayıs University, Faculty of Agriculture, Soil Sciece and Plant Nutrition Department, Samsun Türkiye

Corresponding author email:cgulser@omu.edu.tr

ABSTRACT

The objective of this study was to determine the relationships between field capacity moisture content (FC) and some soil physicochemical properties in 80 surface soil samples (0-20 cm) taken from arable fields around Samsun, Türkiye. After analyzing clay (C), silt (Si), sand (S), organic matter (OM) contents, electrical conductivity (EC), field capacity (FC) and bulk density (BD) of soil samples, FC values were estimated using the other soil properties by linear regression models using stepwise analyses in SPSS program. The FC values varied between 13.80% and 46.00% with a mean of 31.00%. While EC, C, Si, OM contents had significant positive correlations with FC, BD and sand content had negative correlations with FC. To estimate FC value, the multiple linear regressions were produced by stepwise analyses using the soil properties. Producing linear regressions with using only soil textural fractions had lower linear regressions to predict FC. When BD and OM was added into the linear regression models, precision for the estimation of FC increased. According to stepwise analyses, three linear multiple regression models were produced using BD, C, Si and OM contents. While the first model including C and Si contents as variables had 0.876** R^2 and 3.052% RMSE, the second model including BD, C and Si contents had 0.898** R^2 and 2.796% RMSE. The highest R^2 value (0.916**) and the lowest RMSE (2.498%) were estimated with a linear multiple regression model including BD, C, Si and OM contents. It was determined that BD, C, Si and OM contents are most effective soil properties on FC. The FC values of arable fields can be estimated precisely using these soil properties as variables in multiple linear regression models.
Keywords: Soil moisture, multiple regressions, soil texture, bulk density, organic matter.

INTRODUCTION

In sustainable soil management, it is important to know the continuity of soil and water quality which depends on the relationships among soil properties. Soil moisture characteristics should be determined in studies related with soil hydrological properties and plant relations (Rawls, 1982, Puckett et al. 1985). Laboratory measurement of soil moisture characteristic generally takes long time (Rawls, 1982). Multiple linear regressions are used to determine relationships between soil moisture constants and soil properties (Salchow, 1996). The soil properties used in multiple regressions as variables are generally clay, silt sand fractions, EC, soil pH, exchangeable cations, soil organic matter content, bulk density and total porosity (Gülser and Candemir, 2008; Candemir and Gülser, 2012; Gülser and Candemir, 2014; Gülser 2016; Gülser and Ekberli, 2019). Field capacity (CF) defines soil moisture content retained by the soil particles gravimetrically at a pressure of 0.33 bar. Gülser (2004) used pedotransfer functions to determine field capacity and permanent wilting point of cultivated fields. He reported that soil moisture constants can be predicted much more precisely when using soil physical and chemical properties together in the multiple regression models. The objective of this study was to determine the multiple linear regression models between field capacity (FC) values and some soil properties using stepwise analyses to predict moisture content at FC.

MATERIAL AND METHODS

In this study, the relationships between field capacity (FC) moisture contents and some soil physicochemical properties were determined in 80 surface soil samples taken arable fields around Samsun, Türkiye. Some basic soil properties were analyzed as follows; particle size distribution by hydrometer method, bulk density core method (Demiralay, 1993), organic matter (OM) content was determined using the modified Walkley-Black method, electrical conductivity (EC) in the same soil suspension by EC meter, exchangeable cations by ammonia acetate extraction (Kacar, 1994). Field capacity (FC) moisture constants were determined by keeping soil samples saturated for 24 hours on ceramic plates of 1 bar, after than under 0.3 bar pressure until the moisture content of soil samples reached equilibrium (Klute, 1986). Soil moisture contents at FC were taken as dependent variables and stepwise regression models with measured soil properties were created using the SPSS 17.0 program. The accuracy of the multiple regression models were tested with determination coefficient (R^2) and root mean square error (RMSE).

RESULTS AND DISCUSSION

The soil properties, except EC, are generally showed normal distribution (Table 1). According to the EC values, soil samples are generally non-saline, organic matter contents of samples are around moderate level (Soil Survey Staff, 1993). The FC values varied between 13.80% and 46.00% with a mean of 31.00%.

Table 1. Descriptive statistics of soil properties (n=80).

	Minimum	Maximum	Mean	Std. Deviation	Skewness	Kurtosis
Field Capacity, %	13,80	46,00	31,00	8,72	-0,437	-0,894
Clay,%	9,99	68,73	34,71	16,72	0,347	-0,834
Silt,%	3,05	48,74	27,33	9,09	0,127	-0,290
Sand,%	6,84	81,71	37,96	19,73	0,350	-1,017
OM,%	0,58	3,87	1,90	0,73	0,341	-0,259
EC, dS/m	0,11	2,65	0,65	0,46	1,933	5,251
BD, g/cm^3	1,06	1,60	1,39	0,15	-0,498	-0,853

While EC, C, Si, OM contents had significant positive correlations with FC, BD and sand content had negative correlations with FC values. De Macedo et al. (2002) reported that the moisture constants in the multiple regression equations were positively correlated with clay and porosity, and negatively correlated with sand content of soil samples. In another study, Bauer and Black (1992) found significant correlations between the moisture values at field capacity and the organic carbon, sand and clay contents.

To predict FC values, the multiple linear regressions were produced by stepwise analyses using the BD, C, Si and OM contents of soil samples (Table 2). Producing linear regressions with using only soil textural fractions had lower regressions to predict FC. When BD and OM was added into the linear regression models, precision of the FC prediction increased. While the first model including C and Si contents as variables had 0.876** R^2 and 3.052% RMSE, the second model including BD, C and Si contents had 0.898** R^2 and 2.796 RMSE. The highest R^2 (0.916**) value and the lowest RMSE (2.498%) were obtained with a multiple linear regression model including BD, C, Si and OM contents (Figure 1). Gülser (2004) reported that total porosity calculated from the bulk density had the highest direct effect on FC moisture content and using physical properties in pedotransfer models had higher relationships with the measured values compared to chemical properties. He concluded that using physical properties with chemical properties together in the equations increased the accuracy of models increasing R^2 values from 0,706** to 0,853**.

Table 2. Linear multiple regression models used to predict field capacity (FC) values.

Model	Linear Regressions	R^2	RMSE, %
1	FC = 9,163 + 0,464*C + 0,210*Si	0.876**	3,052
2	FC = -26,907 + 0,613*C +0,316*Si + 20,134*BD	0.898**	2,796
3	FC = -36,218 + 0,588*C + 0,301*Si +24,741*BD +2,198*OM	0.916**	2,498

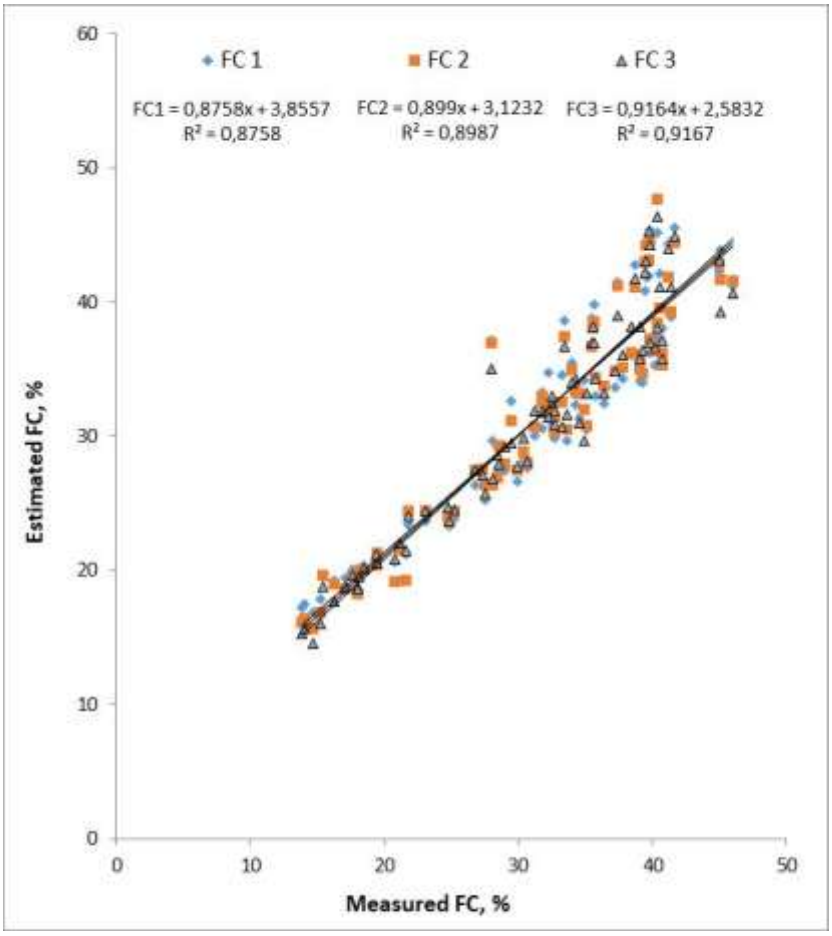


Figure 1. Comparison of measured and estimated field capacity (FC) by three multiple linear regression models.

CONCLUSION

In this study, FC values of the soils had significant positive relationships with EC, C, Si, OM contents, and significant negative relationships with BD and sand contents. It was determined that BD, C, Si and OM contents are most effective soil properties on FC. The FC values of arable fields can be estimated precisely using these soil properties as variables in linear multiple regression models. The equations found in this study can be used to estimate the FC values of agricultural soils in the sampling region having similar properties. However, the success of models in determining moisture characteristics for larger soil groups depends on the results of studies to be conducted with more samples containing greater range for physical and chemical values. Also, the reliability of the models should be examined with other soil sample data set.

REFERENCES

Bauer, A. and Black, A.L. (1992). Organic carbon effects on available water capacity of three soil textural groups. Soil Sci. Soc. Am. J. 56:248-254.

Candemir, F. and C. Gülser, (2012). Influencing Factors and Prediction of Hydraulic Conductivity in Fine Textured-Alkaline Soils. Arid Land Research Management, 26:15-31.

De Macedo, J.R., Meneguelli, N.A., Filho, T.B.O. and Lima, J.A.S. (2002). Estimation of field capacity and moisture retention based on regression analysis involving chemical and physical properties in Alfisols and Ultisols of the state of Rio de Janerio. Commun. Soil Sci. Plant An. 33 (13 and 14): 2037-2055.

Demiralay, İ., 1993. Toprak fiziksel analiz yöntemleri. Atatürk Üniversitesi Ziraat Fakültesi yayınları. Erzurum, 111-120.

Gülser C. and Candemir, F. (2014). Using soil moisture constants and physical properties to predict saturated hydraulic conductivity. Eurasian Journal of Soil Science 3(1): 77 – 81.

Gülser, C. (2004). Tarla kapasitesi ve devamlı solma noktasının toprakların fiziksel ve kimyasal özellikleri ile ilişkili pedotransfer eşitliklerle belirlenmesi. Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi (Anadolu Tarım Bilimleri Dergisi), 19(3), pp.19-23.

Gülser, C. (2016). Predicting aggregate stability of cultivated fields using soil properties. "Spatial Data Processing, Modelling, Analysing and Management for Knowledge Based Systems". International Scientific Conference on Applied Sciences, 27-30 September 2016 - Antalya/Türkiye

Gülser, C. and Ekberli, İ. (2019). Estimating Aggregate Stability by a Linear Regression Model Using Soil Properties. 10th International Soil Congress 2019, Successful Transformation toward Land Degradation Neutrality: Future Perspective" 17-19 June 2019, Ankara, pp:313-317.

Gülser, C. and F. Candemir, (2008). Prediction of Saturated Hydraulic Conductivity Using Some Moisture Constants and Soil Physical Properties. BALWOIS, Ohrid, Republic of Macedonia, 27-31, May. P.328.

Kacar, B. (1994). Bitki ve toprağın kimyasal analizleri: III. Ankara Üniversitesi Ziraat Fakültesi Eğitim, Araştırma ve Geliştirme Vakfı Yayınları No.3, 89-98.

Klute, A. (1986). Water Retention: Laboratory methods. In A. Klute (Ed.) Method of Soil Analysis Part I, Second Edition, Argon. Monog. No 9 ASA Madison WI pp 635-662.

Puckett, W.E., Dane J.H. and Hajek B.F. (1985). Physical and Mineralogical Data to Determine Soil Hydraulic Properties, Soil Sci. Soc. Am. J., 49(4):831–836.

Rawls, W.J., Brakensiek, D.L. and Saxton, K.E. (1982). Estimation of Soil Water Properties, Trans. ASAE, 25(5): 1316-1320.

Salchow, E., Lal, R., Fausey, N.R. and Ward, A. (1996). Pedotransfer functions for variable alluvial soils in southern Ohio. Geoderma 73:165-181.

Soil Survey Staff, 1993. Soil Survey Manuel. USDA Handbook No:18 Washington.

PREDICTING SOIL ELECTRICAL CONDUCTIVITY USING SOME SOIL PROPERTIES

Coşkun GÜLSER

Ondokuz Mayıs University, Faculty of Agriculture, Soil Sciece and Plant Nutrition Department, Samsun Türkiye

Corresponding author email:cgulser@omu.edu.tr

ABSTRACT

In this study, the relationships between soil electrical conductivity (EC) and some soil physicochemical properties were determined in 200 surface soil samples (0-20 cm) taken from cropland fields around Samsun, Türkiye. After analyzing clay, silt, sand, soil reaction (pH), electrical conductivity (EC), organic matter (OM), exchangeable Ca, Mg, K and Na contents of soil samples, regression models using stepwise analyses in SPSS program were done between soil EC values and soil properties. The EC values varied between 150 and 1885 µS/cm with a mean of 616 µS/cm. While exchangeable cations (Ca, Mg, K, Na), pH and clay content had significant correlations with EC, Si and sand contents showed negative correlation with EC. Soil EC values did not show a significant relationship with soil organic matter content. To predict soil EC value, the multiple linear regressions were produced by stepwise analyses using the soil properties. Producing multiple regressions with using only clay, silt, sand, OM and soil pH as variables had lower prediction of EC. When including exchangeable cations in multiple linear regression models, precision of the EC estimation increased. According to stepwise analyses, three multiple linear regression models were produced using only clay, exch. Na and Ca contents. While the first model including just exch. Na as a variable had 0.438** R^2, the second model including exch. Na and clay content as variables had 0.574** R^2. The highest R^2 value (0.595**) and the lowest RMSE (187,28 µS/cm) were estimated with a linear regression including clay and exch. Na and Ca contents. It was determined that clay and exch. Na and Ca contents are the most effective soil properties on predicting of soil EC values.

Keywords: Salt, EC, exchangeable cations, soil texture.

INTRODUCTION

Soluble ion concentration in soil suspension is known as soil salinity which influences soil fertility (Pitman and Läuchli, 2002). Generally, soil salinity level is determined in soil solution via electrical conductivity (EC) measurement. It is an indicator of dissolved ion concentration in soil solution (U.S. Salinity Laboratory Staff, 1954). Gorji et al. (2015) reported that prediction of soil salinity is important to explain soil degradation. Wang et al. (2018) studied several regression models for estimating soil salt content. Multiple linear regressions are generally used for modeling and simulation in most of soil physical, chemical and biological processes researches. Basic soil properties such as clay, silt, sand, organic matter contents, exchangeable cations, total porosity, and bulk density are used as variables for developing multiple regression models (Gülser 2004; Gülser and Candemir, 2008; Candemir and Gülser, 2012; Gülser and Candemir, 2014; Gülser 2016; Gülser and Ekberli, 2019). Benke et al. (2020) reported that most frequently occurring predictors for EC in multiple regression models were soil depth, soil reaction, soil texture and geomorphological mapping unit. In many studies to predict soil EC values, soluble ions in soil solution were used as majajor variables (Shrestha, 2006; Lake et al. 2009; Andrade Foronda and Colinet, 2023). The objective of this study was to predict soil EC values using some soil physical and chemical properties as variables in linear multiple regression models.

MATERIAL AND METHODS

In this study, the relationships between soil electrical conductivity (EC) and some soil physicochemical properties were determined in 200 surface soil samples (0-20 cm) taken from cropland fields around Samsun, Türkiye. Some basic soil properties were analyzed as follows; organic matter (OM) content was determined using the modified Walkley-Black method, particle size distribution by hydrometer method (Demiralay, 1993), soil reaction (pH, 1:1 (w:v) soil:water suspension) by pH meter, electrical conductivity ($EC_{25°C}$) in the same soil suspension by EC meter, exchangeable cations by ammonia acetate extraction (Kacar, 1994). To predict the EC values of soils, linear multiple regression equations between EC and the soil properties were obtained with stepwise analyses using the SPSS statistic program.

RESULTS AND DISCUSSION

Physical and chemical properties of the soil samples used in the study are given in Table 1. The soil samples are generally showed normal distribution, except EC, pH and exch. Na content. According to the EC values of soil samples, 89,1% is none saline, 9,7% is very slightly saline and 1,2% is slightly saline (Figure 1). Soil reaction (pH) of 90% samples is classified as from neutral to moderately alkaline. Organic matter contents are generally classified as low to moderate (Soil Survey Staff, 1993).

Table 1. Descriptive statistics for the properties of soil samples (n=200).

	Minimum	**Maximum**	**Mean**	**Std. Deviation**	**Skewness**	**Kurtosis**
EC, µS/cm	150,00	1885,00	616,39	292,10	1,17	2,11
Clay, %	10,18	68,73	35,56	13,88	0,23	-0,64
Silt, %	3,05	66,37	28,65	9,02	0,53	0,76
Sand, %	4,27	81,71	35,78	16,27	0,55	-0,50
Org. Matter, %	0,20	4,19	1,99	0,75	0,27	-0,15
pH(1:1)	4,85	8,33	7,50	0,61	-1,76	3,36
Ca, cmol/kg	2,36	52,53	21,84	9,74	0,04	-0,49
Mg, cmol/kg	0,52	19,83	6,89	4,20	0,86	-0,09
K, cmol/kg	0,11	1,79	0,58	0,33	0,97	0,64
Na, cmol/kg	0,08	2,52	0,54	0,47	1,80	3,07

Correlations between EC values and soil properties are given in Table 2. Exch. Ca, Mg, K, Na, pH, OM and clay content had significant positive correlations with EC values while sand content had a negative correlation. The correlation values between EC and exch. cations were ordered as follows; Na (0,662**) > K (0,468**) > Mg (0,451**) > Ca (0,332**). In many studies, other researchers determined similar relationships between EC and exch. cation contents (Taghizadeh Mehrjardi et al. 2008; Lake et al. 2009; Candemir and Gülser, 2011; Gülser et al. 2015; Gülser and Candemir, 2015).

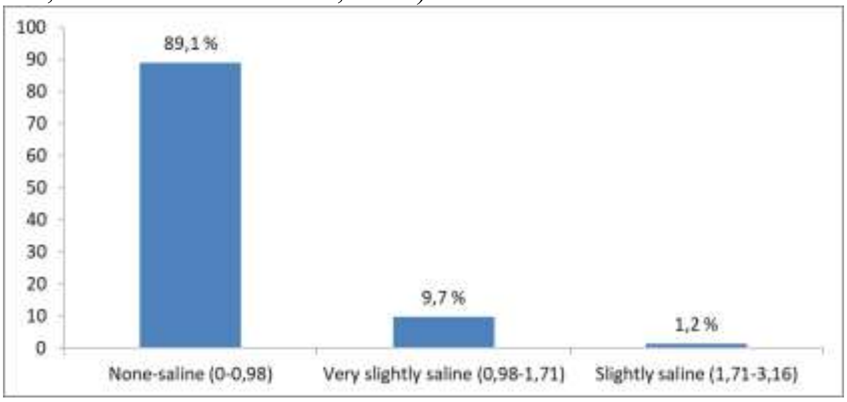


Figure 1. Frequency of EC range (mS/cm) values of the soil samples.

Table 2. The correlation matrix among EC values and soil properties.

	C	Si	S	pH	OM	Ca	Mg	K	Na
EC	0,521**	0,004	-0,447**	0,217**	0,272**	0,332**	0,451**	0,468**	0,662**
C		-0,037	-0,832**	0,561**	0,258**	0,497**	0,403**	0,509**	0,248**
Si			-0,523**	0,060	0,167*	-0,017	0,014	-0,090	0,045
S				-0,512**	-0,313**	-0,414**	-0,351**	-0,384**	-0,237**
pH					0,116	0,395**	0,326**	0,440**	0,015
OM						0,584**	0,488**	0,237**	0,187**
Ca							0,555**	0,522**	0,028
Mg								0,500**	0,345**
K									0,235**

**Correlation is significant at the 0.01 level, *Correlation is significant at the 0.05 level.

To predict soil EC value, the multiple linear regressions were produced by stepwise analyses using the soil properties as variables. Producing multiple linear regressions with using only clay, silt, sand, OM and soil pH as variables had lower relationships to predict EC. When including exchangeable cations in multiple linear regression models, precision of the EC estimation increased. According to stepwise analyses, three linear regression models were produced using only clay, exch. Na and Ca contents as variables (Table 3).

Table 3. Multiple linear regression models used to estimate soil EC values.

Model	Multiple Linear Regressions	R^2	RMSE, µS/cm
1	EC = 352,40 + 416,39*Na	0,438**	219,56
2	EC = 139,74 + 351,77*Na + 8,00*C	0,574**	191,72
3	EC = 88,80 + 362,48*Na + 6,13*C + 5,11*Ca	0,595**	187,27

While the first model including just exch. Na as a variable had 0.438** R^2, the second model including exch. Na and clay content had 0.574** R^2. The highest R^2 value (0.595**) and the lowest RMSE (187,27 μS/cm) were estimated with a multiple linear regression including clay, exch. Na and Ca contents (Figure 2). The comparison of model 1 and model 3 is giving in Figure 2. When using the more soil variable in the multiple linear regression models, the slope of regression line is getting close to 1 and accuracy of the model increasing with reducing RMSE from 219,56 μS/cm to 187,27 μS/cm. Similarly, Candemir and Gülser (2012) reported that using physical and chemical soil properties together in the multiple regression models increased the accuracy and reliability of multiple regressions to estimate soil hydraulic properties.

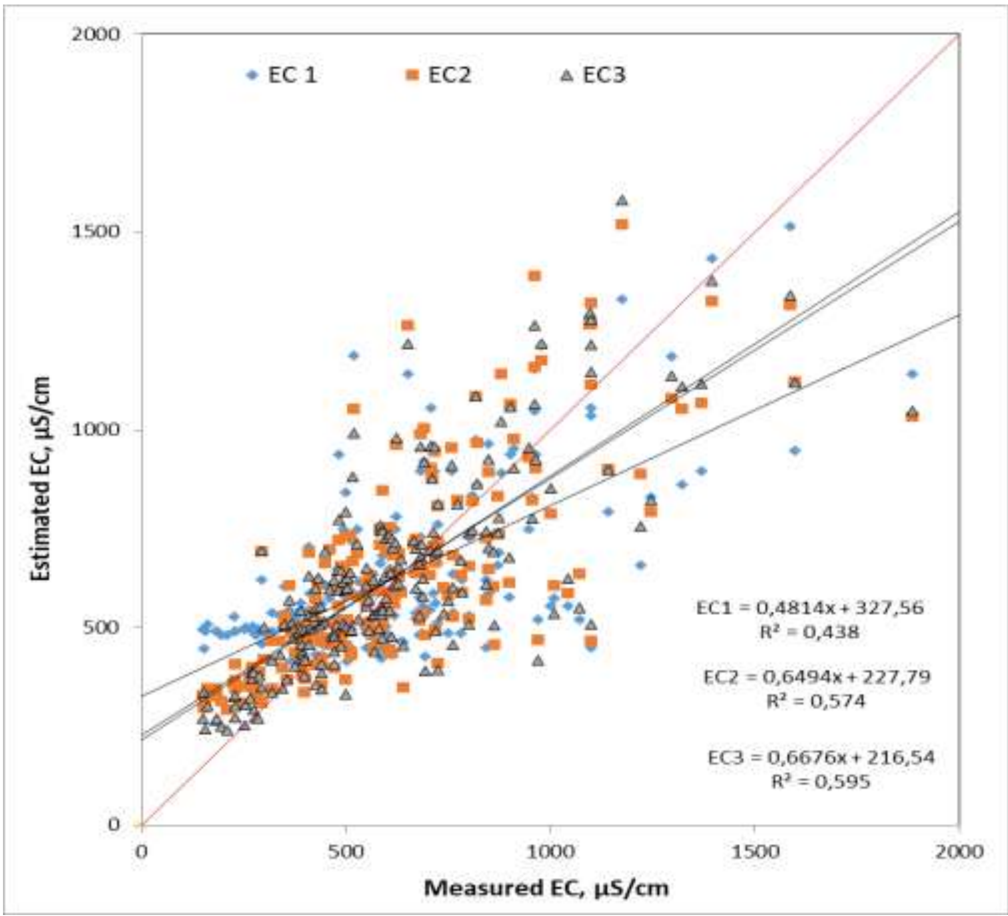


Figure 2. Comparison of measured and estimated EC values by the 3 linear model

CONCLUSION

In this study, EC values of the soils had significant positive relationships with exchangeable cations (Ca, Mg, K, Na), pH, OM and clay content, and a significant negative relationship with sand content. According to the stepwise analyses, it was determined that clay and exch. Na and Ca contents are the most effective soil properties on predicting of soil EC values. When using clay content with exc. cations (Na, Ca) in the multiple regression models, accuracy of the model increased with increasing determination coefficient (R2) and decreasing RMSE values.

REFERENCES

Andrade Foronda, D. and Colinet, G., (2023). Prediction of soil salinity/sodicity and salt-affected soil classes from soluble salt ions using machine learning algorithms. Soil Systems, 7(2), p.47.

Benke, K.K., Norng, S., Robinson, N.J., Chia, K., Rees, D.B. and Hopley, J. (2020). Development of pedotransfer functions by machine learning for prediction of soil electrical conductivity and organic carbon content. Geoderma, 366, p.114210.

Candemir, F. and Gülser, C. (2012). Influencing Factors and Prediction of Hydraulic Conductivity in Fine Textured-Alkaline Soils. Arid Land Res. Management, 26:15-31.

Candemir, F. and Gülser, C. (2011). Effects of different agricultural wastes on some soil quality indexes at clay and loamy sand fields. Comm. Soil Sci. Plant Analy. 42 (1):13-28.

Demiralay, İ. (1993). Toprak fiziksel analiz yöntemleri. Atatürk Üniversitesi Ziraat Fakültesi yayınları. Erzurum, 111-120.

Gorji, T., Tanik, A. and Sertel, E. (2015). Soil salinity prediction, monitoring and mapping using modern technologies. Procedia Earth and Planetary Science, 150, pp.507-512.

Gülser C. and Candemir, F. (2014). Using soil moisture constants and physical properties to predict saturated hydraulic conductivity. Eurasian Journal of Soil Science 3(1): 77 – 81.

Gülser C., Candemir F. (2015). Effects of agricultural wastes on the hydraulic properties of a loamy sand cropland in Turkey. Soil Science & Plant Nutrition 61 (3): 384-391.

Gülser C., Kızılkaya, R., Aşkın, T., Ekberli , İ. (2015). Changes in Soil Quality by Compost and Hazelnut Husk Applications in a Hazelnut Orchard. Compost Science and Utilization, 23:3, 135-141.

Gülser, C. (2016). Predicting aggregate stability of cultivated fields using soil properties. "Spatial Data Processing, Modelling, Analysing and Management for Knowledge Based Systems". International Scientific Conference on Applied Sciences, 27-30 September 2016 - Antalya/Türkiye

Gülser, C. (2004). Tarla kapasitesi ve devamlı solma noktasının toprakların fiziksel ve kimyasal özellikleri ile ilişkili pedotransfer eşitliklerle belirlenmesi. Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi (Anadolu Tarım Bilimleri Dergisi), 19(3), pp.19-23.

Gülser, C., Ekberli, İ. (2019). Estimating Aggregate Stability by a Linear Regression Model Using Soil Properties. 10th International Soil Congress 2019, Successful Transformation toward Land Degradation Neutrality: Future Perspective" 17-19 June 2019, Ankara, pp:313-317.

Gülser, C. and Candemir, F. (2008). Prediction of Saturated Hydraulic Conductivity Using Some Moisture Constants and Soil Physical Properties. BALWOIS, Ohrid, Republic of Macedonia, 27-310, May. P.328.

Kacar, B. (1994). Bitki ve toprağın kimyasal analizleri:III. Ankara Üniversitesi Ziraat Fakültesi Eğitim, Araştırma ve Geliştirme Vakfı Yayınları No.3, 89-98.

Lake, H.R., Akbarzadeh, A. and Mehrjardi, R.T. (2009). Development of pedotransfer functions (PTFs) to predict soil physico-chemical and hydrological characteristics in southern coastal zones of the Caspian Sea. Journal of Ecology and the Natural Environment, 1(7), pp.160-172.

Pitman, M.G. and Läuchli, A. (2002). Global impact of salinity and agricultural ecosystems. Salinity: environment-plants-molecules, 30, p.20.

Shrestha, R.P. (2006). Relating soil electrical conductivity to remote sensing and other soil properties for assessing soil salinity in northeast Thailand. Land degradation & development, 17(6), pp.677-689.

Soil Survey Staff, (1993). Soil Survey Manuel. USDA Handbook No:18 Washington.

U.S.Salinity Laboratory Staff, (1954). Diagnosis and improvement of saline and alkali soils. Agriculture handbook, 60, pp.83-100.

Wang, X., Zhang, F., Ding, J., Latif, A. and Johnson, V.C. (2018). Estimation of soil salt content (SSC) in the Ebinur Lake Wetland National Nature Reserve (ELWNNR), Northwest China, based on a Bootstrap-BP neural network model and optimal spectral indices. Science of the Total Environment, 615, pp.918-930.

FACTORS CAUSING BIODIVERSITY LOSS AND THE ROLE OF HEAVY METALS IN BIODIVERSITY LOSS

Burak ÖZDEMİR[1], Füsun GÜLSER[2], Siyami KARACA[2], Bulut SARĞIN[2]

[1]*Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Field Crops*
[2]*Van Yüzüncü Yıl University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition*

Corresponding author e-mail: f.gulser@yyu.edu.tr

ABSTRACT

Global biodiversity loss and mass extinction of species are two of the most critical environmental problems the world currently faces. Loss of biodiversity causes the degradation of various ecosystems that are of central importance to the ecological cycle and human health. It is our responsibility to preserve biodiversity as effectively as possible. It has been stated in research conducted by scientists in recent years that if the loss of biodiversity is ignored, it will have consequences that may even threaten the existence of the human race. In new studies, it is necessary to understand the main reasons responsible for the loss of various species on our planet. One of the many factors causing biodiversity loss is heavy metal pollution. Among various pollutants, heavy metals are of concern due to their environmental persistence, biogeochemical recycling and ecological risks. They are considered among the most dangerous environmental pollutants because they do not break down through physical processes and therefore remain in nature for a long time. Through the process of biomagnification and bioaccumulation, heavy metals have a significant impact on biological systems. Therefore, heavy metal pollution can cause a decrease in pollination in certain populations, difficulties in breeding or feeding certain species, and changes in the physiology, anatomy of organisms and biotope characteristics of local populations. In this study, the factors that cause the decrease in biodiversity and the negative effects of heavy metal pollution on biodiversity were discussed.

Keywords: Biodiversity loss, heavy metal pollution, soil pollution, climate change

CONCEPT OF BIODIVERSITY

Biodiversity is the diversity of different life forms on Earth, including various plants, animals, microorganisms, their genetic makeup, and the ecosystems they create. It refers to the genetic diversity, ecosystem diversity, and species diversity (the number of species) within an area, biome, or planet. Biodiversity is vital in various ways, such as promoting the aesthetic value of the natural environment and contributing to our material well-being through utilitarian values by providing food, feed, fuel, timber, and medicine. Biodiversity is a life-support system. Organisms depend on air to breathe, food to eat, and water to drink. Wetlands filter pollutants from water, trees and plants absorb carbon to reduce global warming, and bacteria and fungi decompose organic matter to enrich the soil. It has been experimentally shown that local species richness is linked to the health of ecosystems and is connected to human quality of life. The ecosystem services of biodiversity are maintained through soil formation and conservation, water conservation and purification, sustaining hydrological cycles, regulating biochemical cycles, absorbing and breaking down pollutants and waste materials through decomposition, and determining and regulating the natural world's climate. Despite the benefits of biodiversity, threats to species and ecosystems are increasing at an alarming rate today, most of which stem from the mismanagement of biological resources by humans, often driven by reckless economic policies, pollution, and faulty institutions, in addition to climate change. It is essential to protect biodiversity to ensure intergenerational equity. Some of the current biodiversity conservation measures include afforestation, zoos, botanical gardens, national parks, biosphere reserves, germplasm banks, and the adoption of breeding techniques, tissue culture techniques, and social forestry to minimize the stress on forest resource exploitation.

BİODİVERSİTY LOSS

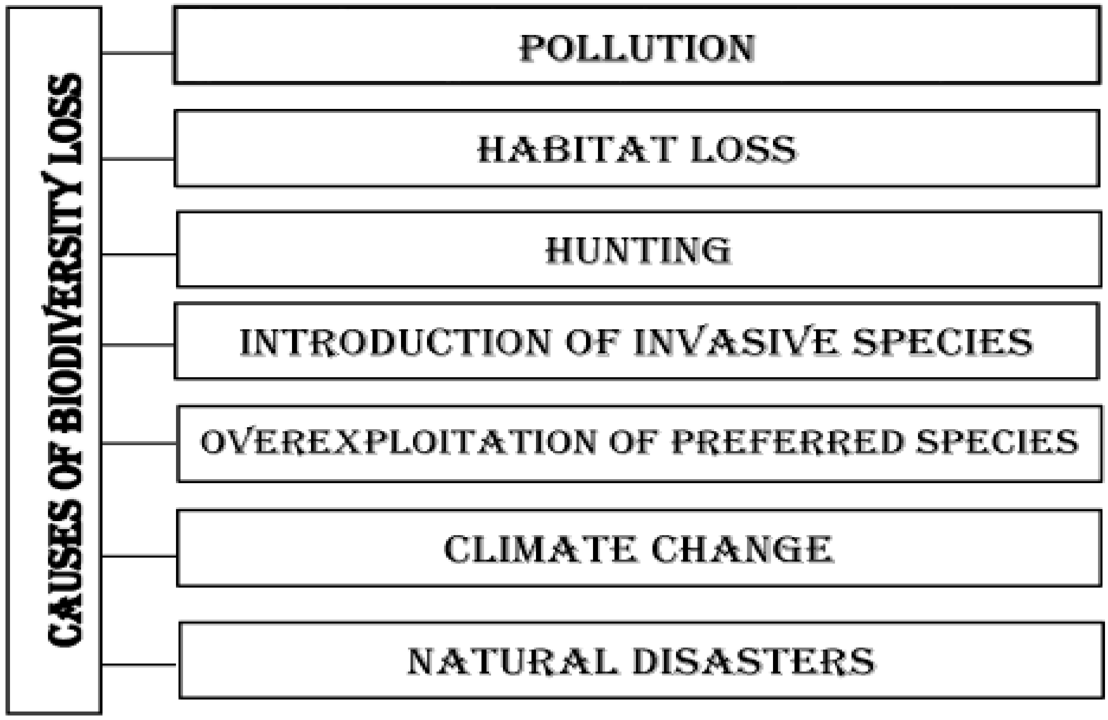
Humans are now a dominant force in the restructuring of the Earth's biosphere (Vitousek et al. 1997; Dobson 2005). With a growing human population of 90 million each year, it could reach 9 billion or more by the middle of the twenty-first century (Cohen 1995, 2005). Humans are rapidly overconsuming natural resources, disproportionately utilizing a significant portion of the Earth's primary production, and transforming natural habitats into human-dominated landscapes (for example, Imhoff et al. 2004). The environmental impacts of human activities are evident worldwide, encompassing striking changes in species composition, abundance, and organism diversity in various ecosystems (Biesmeijer et al. 2006). The unprecedented changes in biodiversity described by Dirzo and Raven (2003) as the sixth great extinction in the history of life on Earth include both the global-scale extinction of species (Mittermeier et al. 2000; Myers et al. 2000) and the loss (i.e., extinction) and introduction of species at a smaller, more local scale (Ehrlich 2004). Such changes contribute to biotic homogenization, which could have significant secondary economic effects (an increase in the similarity of biological diversity patterns between locations) (Olden and Rooney 2006). Biodiversity has been declining at a concerning rate in recent years. According to Van Klink et al. (2020), terrestrial insect abundance is showing a similarly alarming rate of decline.

The human species, like other species, cannot survive independently of its interactions with other organisms. Edward O. Wilson coined the term biophilia to describe humans' "innate tendency to be attracted to other forms of life and to connect with natural living systems". Biophilia means "human dependence on nature" (Kellert and Wilson 1993), and the fate of other species cannot be considered completely separate from our own sustainability. The diversity of living plants, animals, and microorganisms is a fundamental resource for humans because other organisms provide food, medicine, clean water and air, recreational spaces, and other ecosystem services (e.g., Daily 1997 Kremen 2005). We cannot afford to continuously lose our resources and ecological partners, as their loss jeopardizes the stability of ecosystem services and our ecological life support systems (Rosenzweig 2003). In the face of rapidly increasing human population and associated global environmental changes, there is a need for innovative strategies to ensure the conservation and preservation of biodiversity on our planet (Dower et al. 1997).

As the human population increases and economic development occurs in a specific geographical area, the human-induced transformation of ecosystems often destroys natural habitats and biodiversity, and also alters ecosystem services, regardless of the consequences of losses in the near and distant future (Balmford et al. 2002). At both global and local scales, humanity is compelled to redirect demographic and land use patterns to avoid ongoing negative changes in local and global biodiversity and ecosystems, that is, to prevent the irreversible destruction of our life support systems. This challenge requires the participation of each of us who is concerned about the future of humanity and the biosphere (for example, Ehrlich and Wilson 1991; Brown 2006).

FACTORS CONTRİBUTİNG TO THE DECLİNE OF BİODİVERSİTY

The decline of biological diversity (Figure 1) is attributed to numerous factors such as pollution, habitat loss, hunting, the introduction of invasive species, overexploitation of preferred species, climate change, and natural disasters.



1. Habitat Loss

Habitat and wildlife are causally related. We cannot risk neglecting wildlife to obtain the benefits we rely on for the active functioning of the ecosystem. The degradation, deterioration, and fragmentation of habitats are the three dominant categories of habitat loss (Klappenbach, 2020).

Habitat degradation refers to severe damage to a species' natural habitat to the extent that it can no longer support local ecosystems and species. This ultimately leads to the extinction of species, which means the loss of biological diversity. Examples of habitat degradation include deforestation for agricultural purposes, filling wetlands, clearing land for residential or commercial areas, harvesting fossil fuels, and so on (Martins et al., 2018).

Habitat fragmentation is another massive issue that arises due to human development activities. Humans transform vast wild areas into smaller land parcels to meet their endless needs for development, even at the cost of their own species' extinction. These fragmented areas disrupt the living spaces of animal and plant species, isolate animal populations, and compress genetic diversity (Bright, 1993).

2. Hunting

Hunting is a primary cause of the extinction of many animals within the food web. As a result, various species in the region are negatively affected due to facing food shortages or a complete lack of food compared to normal conditions. Hunting activities impose a significant burden on wildlife, leading to a substantial decline in wildlife populations and resulting in degraded and unproductive ecosystems. Overhunting can lead to the disappearance of large mammals in healthy and undisturbed habitats (Symes et al.,2018).

3. Overexploitation of Certain Species

Overexploitation refers to the collection of species from their natural habitats at rates higher than local communities can sustainably recover. Currently, approximately one-third of the world's vertebrates facing the threat of extinction are vulnerable due to overexploitation. Overfishing and hunting are examples of overexploitation. The once-abundant passenger pigeons are victims of excessive hunting (Hammerschlag and Gallagher, 2017).

The overharvesting of plants with medicinal value has led to their disappearance from natural habitats. Many plants, such as Drosera sp., Gnetum sp., pitcher plants, Psilotum sp., and Nepenthes khasiana, are relentlessly hunted and collected for academic training and laboratory experiments. Similarly, various orchids have also been overexploited (Bodeker, 2014).

4. Climate Change

Biodiversity and climate change are strongly interconnected. Although climate has continuously changed throughout the entire history of the world, along with the evolution and extinction of ecological communities and species, the accelerating climate change disrupts ecological systems and the ability of species to adapt, thereby increasing the loss of biodiversity. Rapid climate change, which promotes biodiversity loss, jeopardizes the security of clean water, air, and the natural resources we depend on. It will be difficult to access these natural resources due to the reduction or extinction of the flora and fauna from which they are derived. Climate change emerges as a significant threat to biodiversity on Earth, alongside other factors such as habitat loss, land degradation, hunting, and the overexploitation of certain species (Linders et al. 2019).

5. Invasive Species

The introduction of invasive species poses a significant threat to the biodiversity crisis. Invasive species are those that are not native to the ecosystem, often arriving or being introduced through human activities, and begin to reproduce. These species are harmful because they disproportionately affect the ecosystem compared to other species. Most of the new species introduced to an ecosystem do not become invasive; however, a few do turn into invasive species and negatively impact the ecosystem. Invasive species disrupt local ecosystems in many ways, such as altering habitats, importing pathogens, grazing on local plants, hybridizing with native species leading to a reduction in genetic diversity, and preying on local species for resources (Kilpatrick et al. 2017).

6. Natural Disasters

Natural disasters such as volcanoes, wildfires, floods, hurricanes, droughts, epidemics, tsunamis, etc., lead to a significant loss of biodiversity. Flooding is common in humid tropical regions like Central Africa and eastern and northern Australia, as well as in some areas of South America. Tropical regions host a vast amount of vegetation and animal species. Flooding washes away large quantities of nutrients from the soil, while drought causes the soil to dry out and lowers groundwater levels. In this situation, both animals and plants are harmed. Similarly, wildfires in dense forested areas and earthquakes significantly disrupt the lives of organisms, thereby affecting biodiversity. Volcanoes often destroy animals and plants in adjacent areas. The occurrence of epidemics in nature typically remains limited to a specific animal or plant population, as the pathogens are usually species-specific, resulting in the extinction of a large majority of that population (Chapin and Diaz, 2020)

7. Pollution

Living organisms have evolved over a long period, yet they struggle to adapt to life on a polluted planet.

7.a) Air Pollution

Air pollutants such as soot, dust, ammonia, and carbon dioxide can directly and indirectly affect biodiversity. Air pollution affects the respiratory apparatus of animals and negatively affects their welfare, including egg-laying ability and behavioural changes (Sanderfoot and Holloway, 2017) Air pollution is also known to affect the reproductive ability of animals and thus reproductive success. The massive release of greenhouse gases such as nitrous oxide, carbon dioxide and methane is rapidly changing the earth's climate. Acid rain is another pollutant and causes increased mucus in fish gills leading to suffocation. Soil prone to acid rain has reduced the activity of microbes (Pennanen et al., 1998). This also affects the food chain and other forms of life.

7.b) Water Pollution

Nitrogen and phosphorus contained in chemical fertilisers added to the soil to increase crop productivity are transported from the soil to water bodies or underground. The presence of these nutrients in water bodies leads to eutrophication or excessive plant growth. Eutrophication leads to reduced oxygen levels, which is detrimental to biodiversity, and due to lack of oxygen, fish and other aquatic animals die. Like fertilisers, pesticides can also accumulate in water bodies. Pesticides adversely affect non-flowing water bodies such as lakes and ponds as fertilisers are not washed away and animals in water bodies have difficulty in reproduction (Bhateria and Jain, 2016).

Various anthropogenic activities such as cement, car production; mining, etc. lead to the entry of heavy metals such as arsenic, cadmium, mercury into water bodies [9]. Heavy metals affect the behaviour and survival rates of aquatic animals, especially fish. In addition, events such as oil spills greatly affect wildlife, especially in deep oceans, causing impaired animal senses, drowning, disruption of vital organs of organisms, reduced growth rates and higher mortality rates of larvae. Plastic that remains in the environment for longer periods of time also causes internal injuries leading to death in animals, especially turtles. It has been noticed that seabirds such as the Layson's albatross die before flying from the nest after consuming plastic. (Wright et al. 2013).

7.c) Soil pollution

Soil pollution is another factor that negatively affects biodiversity. Excessive use of fertilisers, pesticides and antibiotics used in agriculture is also very harmful for biodiversity. These agricultural pollutants, such as nitrogen in fertilisers, change the pH and nutrient level of the soil. The increased presence of nutrients in the soil causes weed species to grow strongly and leads to the inhibition of the growth of wild flowers, which are essential for bees and other pollinating insects (Klein et al., 2007). This greatly affects biodiversity. Soil contaminated with heavy metals greatly affects the well-being of microorganisms, which are essential for the sustainable life of living organisms. The excess of heavy metals in soil is not easily degraded and accumulated by plants (Singh et al., 2015).

Heavy Metal Pollution

One of the many factors causing biodiversity loss is heavy metal pollution. Among various pollutants, heavy metals are of concern due to their environmental persistence, biogeochemical recycling and ecological risks. They are considered among the most dangerous environmental pollutants because they do not break down through physical processes and therefore remain in nature for a long time. Therefore, heavy metal pollution can cause a decrease in pollination in certain populations, difficulties in breeding or feeding certain species, and changes in the physiology, anatomy of organisms and biotope characteristics of local populations.

Heavy metals can be emitted into the environment through natural resources and anthropogenic activities; anthropogenic activities are the main causes of emission. Among them, mining activities represent the greatest threat to ecosystem integrity due to the persistence of heavy metals in the environment, which persist for hundreds of years after the end of mining activities (Duruibe et al., 2007)). In environmental exposures, these toxic substances show their effects in the following ways Different mechanisms, chronic exposures at low doses of complex metal mixtures are responsible for the effects observed in wild animal populations and communities, and have ecosystem-level implications (Mussali-Galante et al., 2014). Therefore, this type of exposure poses a threat to biodiversity.

Exposed individuals integrate exposure to the pollutants in their environment and react in a measurable and predictable way; these reactions are observed at different levels of biological organization (Bickham et al., 2000). Therefore, it is necessary to use biological markers or biomarkers to better understand the ecological consequences of metal exposure. Biomarkers are tools that allow the degree of exposure and the effects of environmental chemical contamination to be analyzed (Handy et al., 2003). These measurements offer valuable estimators of ecologically relevant impacts. However, in ecotoxicology, where exposed populations, communities and ecosystem-level consequences are the focus of attention, the use of biomarkers is not an easy task, as responses to toxic chemical stress become less specific and many variables interfere with physiological responses. In this context, Bickham and colleagues explain that although the damage caused by xenobiotic exposure is at the cellular or genetic levels, the effects can be observed at higher levels of biological organization (emergent effects) (Bickham et al., 1994). Especially if biomarkers are to be used as indicators of action, it is important to take into account that the biomarker response should be tightly and regularly linked to the responses at these higher levels (Forbes et al., 2006). Also, biomarkers of exposure (external dose, internal dose; bioaccumulation levels), biomarkers of biological effective dose (DNA adducts) and biomarkers of effect (DNA breaks) must be used to analyze the relationship between the cellular and genetic effects with ecological responses.

At the population level, some considerations should be taken into account when analyzing the resulting effects. Some of these effects are: changes in sex ratios, changes in age structure, low reproductive success, inbreeding, changes in genetic structure and diversity, low fitness and population declines (Mussali-Galante et al., 2013). However, these effects are not specific to environmental metal exposures. Therefore, differences in biomarker response between populations of a species can be carefully addressed by analyzing geographical influences, habitat influence, population fragility (specific to the population in question) and exposure history (Forbes et al., 2006). The differences in biomarker response between populations of a species can be carefully Deciphered. In the last decade, one of the emerging effects evaluated in environmentally exposed populations is changes in genetic pools, which have been identified by Mussali-Galante and collaborators as persistent biomarkers (Mussali-Galante et al., 2014).

At the community level: Changes in diversity and species richness, changes in dominant species, changes in species composition, and biodiversity loss may be some of the emerging effects. However, due to the complexity of species interactions, as was realized many years

ago, such effects cannot be accurately predicted from population-level effects (Hopkin, 1993; Lagadic et al., 1994).
Studies evaluating community-level responses to environmental metal stress are mostly conducted in aquatic ecosystems using invertebrate and fish communities. Among the few studies conducted in terrestrial ecosystems, insect communities are the focus of interest (Nahmani et al., 2014). In such studies, the bioaccumulation levels in different invertebrate groups are analyzed and the relationship between bioaccumulation levels and community effects (especially species richness and composition) is examined.
At the ecosystem level, biomagnification (bioaccumulation at successive trophic levels) is well documented for some metals. Trophic chain effects have been observed in which individuals fed at lower levels in the food chain are usually exposed to lower metal concentrations. In such studies, primary producers (plants) represent an important step in metal transfer, as they form the basis of the food chain. Therefore, some metals can be transported from plants to higher layers of the food chain, posing a threat to biodiversity and ecosystem integrity (Peralta-Videa et al., 2009).
Chronic environmental metal exposures exert their negative effects on individuals health, having consequences at the population and community levels, putting ecosystem integrity at risk. However, the recognition and use of biomarkers in ecotoxicology has been a difficult task, due to the unspecific responses and multiple variables that affect physiological responses to toxic. Therefore, it becomes necessary that ecotoxicological studies include: HM concentrations in soils, bioaccumulation parameters in vertebrate and invertebrate species, the relationship between these biomarkers with morphological, anatomic and physiological alterations that may alter population parameters. In particular, the use of bioindicator or sentinel species is necessary in order to evidence the consequences of HM exposure in wild populations.

CONCLUSİONS AND PERSPECTİVES

It is essential to protect biodiversity to ensure intergenerational equity. Some of the current biodiversity conservation measures include afforestation, zoos, botanical gardens, national parks, biosphere reserves, germplasm banks, and the adoption of breeding techniques, tissue culture techniques, and social forestry to minimize the stress on forest resource exploitation.
Chronic environmental metal exposures have negative effects on the health of individuals, have consequences at the population and community level, and put ecosystem integrity at risk. However, the recognition and use of biomarkers in ecotoxicology has been a difficult task due to non-specific responses and multiple variables affecting physiological responses to toxicants. Therefore, it becomes necessary for ecotoxicological studies to include the following: HM concentrations in soil, parameters of bioaccumulation in vertebrate and invertebrate species, the relationship of these biomarkers to morphological, anatomical and physiological changes that may change population parameters. In particular, it is necessary to use bioindicator or sentinel species to prove the consequences of HM exposure in wild populations.
Terrestrial invertebrates have been used as an ideal system to assess community responses to environmental chemical stress due to their easy capture, wide distribution, abundance, low mobility and close contact with HM from the soil. Especially earthworms and arthropods are the most studied organisms. In contrast, studies assessing the effects of HM on vertebrate community structure are rare, probably due to their body size, mobility and sampling difficulties. However, when working with vertebrates, the study of small mammal species, which serve as good bioindicators, has been an excellent alternative and the results can be easily compared with humans. Furthermore, a methodological strategy in many studies has been the use of pollution gradients to visualise subtle changes in HM concentrations along a soil gradient

and to relate these changes to some community structure parameters. At present, we can conclude that HM differentially affects the community structure and community functioning of the different animal groups studied so far.

At the community level, the search for new biomarkers continues. In this context, abundance changes in different guilds that fit into the community can also be used as biomarkers, since changes in abundance or disappearance of guilds in exposed communities may serve as an ecological response to chemical stress.

At the ecosystem level, ecotoxicological studies are very limited. Trophic chain alterations, biomagnification and modifications in nutrient and energy cycles have been reported. Studies generally asses HM transfer along three trophic levels, such studies have concluded that metal flux depends on the biology of the species, on the trophic position in the chain and on the metal type or metal mixture in question. Mainly, HM transfer from plants to invertebrate herbivores (insects) and from insects to other invertebrates (spiders) or predator vertebrates (small mammals) has been the point of interest. The information from these studies has gained attention, especially because human beings represent the last level of the trophic chain, such as in the case of agroecosystems. It is desirable to use as biomarkers in ecosystem studies, measures of stable carbon and nitrogen isotopes for evaluation of HM transfer along terrestrial trophic chains.

Ecotoxicological studies at the ecosystem level are very limited. Trophic chain changes, biomagnification and modifications in nutrient and energy cycles have been reported. Studies generally assess HM transfer across three trophic levels, with such studies concluding that metal flux depends on the biology of the species, the trophic position in the chain and the metal type or metal mixture in question. Of primary interest has been HM transfer from plants to invertebrate herbivores (insects) and from insects to other invertebrates (spiders) or predatory vertebrates (small mammals). The information obtained from these studies has attracted attention, especially since humans represent the last level of the trophic chain, as in agricultural ecosystems. It is desirable to use biomarkers, measures of stable carbon and nitrogen isotopes, in ecosystem studies to assess HM transfer along terrestrial trophic chains.

Finally, as a result of biomarker approaches, future efforts need to integrate different biological and ecological responses at all levels of biological organization. Furthermore, study designs should be more rigorous, including multiple species and multiple biomarkers, allowing for more realistic assessment of HM exposure, which will allow for better prediction, understanding, and resolution of HM pollution problems worldwide.

REFERENCES

Balmford, A., Bruner, A., Cooper, P., Costanza, R., Farber, S., Green, R. E., ... & Turner, R. K. (2002). Economic reasons for conserving wild nature. science, *297*(5583), 950-953.

Bhateria, R., & Jain, D. (2016). Water quality assessment of lake water: a review. *Sustainable water resources management*, *2*, 161-173.

Bickham, J. W., Sandhu, S., Hebert, P. D., Chikhi, L., & Athwal, R. (2000). Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. *Mutation research/Reviews in Mutation research*, *463*(1), 33-51.

Biesmeijer, J. C., Roberts, S. P., Reemer, M., Ohlemuller, R., Edwards, M., Peeters, T., ... & Kunin, W. E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science, 313*(5785), 351-354.

Bodeker, G., van ‘t Klooster, C., & Weisbord, E. (2014). Prunus africana (Hook. f.) Kalkman: the overexploitation of a medicinal plant species and its legal context. *The Journal of Alternative and Complementary Medicine*, *20*(11), 810-822.

Bright, P. W. (1993). Habitat fragmentation-problems and predictions for British mammals. *Mammal Review*, *23*(3-4), 101-111.

Brown, L. R. (2006). Rescuing a planet under stress. *The Futurist*, *40*(4), 18.

Chapin III, F. S., & Díaz, S. (2020). Interactions between changing climate and biodiversity: Shaping humanity’s future. *Proceedings of the National Academy of Sciences*, *117*(12), 6295-6296.

Cohen, J. E. (1995). How many people can the earth support?. *The sciences*, *35*(6), 18-23.

Daily, G. R. (1998). Nature's services: societal dependence on natural ecosystems. *Environmental Values*, *7*(3).

Dirzo, R., & Raven, P. H. (2003). Global state of biodiversity and loss. *Annual review of Environment and Resources*, *28*(1), 137-167.

Dobson, A. (2005). Monitoring global rates of biodiversity change: challenges that arise in meeting the Convention on Biological Diversity (CBD) 2010 goals. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *360*(1454), 229-241.

Dower, R., & Ditz, D. (1998). *Frontiers of sustainability: environmentally sound agriculture, forestry, transportation, and power production* (No. ISLAND-0112/XAB). Island Press, Washington, DC (United States).

Duruibe, J. O., Ogwuegbu, M. O. C., & Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. *International Journal of physical sciences*, *2*(5), 112-118.

Ehrlich, P. R., & Ehrlicuh, A. H. (2004). Collision course: population, profligacy, power and the struggle for sustainability. *Island, Washington*.

Ehrlich, P. R., & Wilson, E. O. (1991). Biodiversity studies: science and policy. *Science*, *253*(5021), 758-762.

Forbes, V. E., Palmqvist, A., & Bach, L. (2006). The use and misuse of biomarkers in ecotoxicology. *Environmental Toxicology and Chemistry: An International Journal*, *25*(1), 272-280.

Hammerschlag, N., & Gallagher, A. J. (2017). Extinction risk and conservation of the earth's national animal symbols. *BioScience*, *67*(8), 744-749.

Handy, R. D., Galloway, T. S., & Depledge, M. H. (2003). A proposal for the use of biomarkers for the assessment of chronic pollution and in regulatory toxicology. *Ecotoxicology*, *12*, 331-343.

Hopkin, S. P. (1993). Ecological implications of'95% protection levels' for metals in soil. *Oikos*, 137-141.

Imhoff, M. L., Bounoua, L., Ricketts, T., Loucks, C., Harriss, R., & Lawrence, W. T. (2004). Global patterns in human consumption of net primary production. *Nature*, *429*(6994), 870-873.

Kellert, S. R., & Wilson, E. O. (1993). The Biophilia Hypothesis (Island Pre). *Washington, DC*.

Kilpatrick, A. M., Salkeld, D. J., Titcomb, G., & Hahn, M. B. (2017). Conservation of biodiversity as a strategy for improving human health and well-being. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1722), 20160131.

Klein, A. M., Vaissière, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. *Proceedings of the royal society B: biological sciences*, *274*(1608), 303-313.

Kremen, C. (2005). Managing ecosystem services: what do we need to know about their ecology?. *Ecology letters*, *8*(5), 468-479.

Lagadic, L., Caquet, T., & Ramade, F. (1994). The role of biomarkers in environmental assessment (5). Invertebrate populations and communities. *Ecotoxicology*, *3*, 193-208.

Linders, T. E. W., Schaffner, U., Eschen, R., Abebe, A., Choge, S. K., Nigatu, L., ... & Allan, E. (2019). Direct and indirect effects of invasive species: Biodiversity loss is a major mechanism by which an invasive tree affects ecosystem functioning. *Journal of Ecology*, *107*(6), 2660-2672.

Martins, I. S., Rosa, I., & Pereira, H. M. (2018). Projecting impacts of global land-use scenarios on biodiversity change across scales and species groups. In *ECCB2018: 5th European Congress of Conservation Biology. 12th-15th of June 2018, Jyväskylä, Finland*. Open Science Centre, University of Jyväskylä.

Mittermeier, R. A., Myers, N., Gill, P. C., & Mittermeier, C. G. (2000). Hotspots: Earth's richest and most endangered terrestrial ecoregions. *Mexico City: CEMEX*.

Mussali-Galante, P., Tovar-Sánchez, E., Valverde, M., & Rojas, E. (2014). Genetic structure and diversity of animal populations exposed to metal pollution. *Reviews of Environmental Contamination and Toxicology, Volume 227*, 79-106.

Myers, N., Mittermeier, R. A., Mittermeier, C. G., Da Fonseca, G. A., & Kent, J. (2000). Biodiversity hotspots for conservation priorities. *Nature*, *403*(6772), 853-858.

Nahmani, J., & Lavelle, P. (2002). Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. *European Journal of Soil Biology*, *38*(3-4), 297-300.

Olden, J. D., & Rooney, T. P. (2006). On defining and quantifying biotic homogenization. *Global Ecology and Biogeography*, *15*(2), 113-120.

Pennanen, T., Fritze, H., Vanhala, P., Kiikkilä, O., Neuvonen, S., & Bååth, E. (1998). Structure of a microbial community in soil after prolonged addition of low levels of simulated acid rain. *Applied and Environmental Microbiology*, *64*(6), 2173-2180.

Rosenzweig, M. L. (2003). *Win-win ecology: how the earth's species can survive in the midst of human enterprise*. Oxford University Press.

Sanderfoot, O. V., & Holloway, T. (2017). Air pollution impacts on avian species via inhalation exposure and associated outcomes. *Environmental Research Letters*, *12*(8), 083002.

Singh, V., Tripathi, B. N., & Sharma, V. (2015). Interaction of Mg with heavy metals (Cu, Cd) in T. aestivum with special reference to oxidative and proline metabolism. *Journal of plant research*, *129*, 487-497.

Symes, W. S., Edwards, D. P., Miettinen, J., Rheindt, F. E., & Carrasco, L. R. (2018). Combined impacts of deforestation and wildlife trade on tropical biodiversity are severely underestimated. *Nature communications*, *9*(1), 4052.

Wright, S. L., Rowe, D., Thompson, R. C., & Galloway, T. S. (2013). Microplastic ingestion decreases energy reserves in marine worms. *Current Biology*, *23*(23), R1031-R1033.

Vitousek, P. M., Mooney, H. A., Lubchenco, J., & Melillo, J. M. (1997). Human domination of Earth's ecosystems. *Science*, *277*(5325), 494-499.

FLIGHT ALTITUDE AND CAMERA RESOLUTION CHARACTERISTICS OF UAV IMAGES IN ENDEMIC PLANT DETECTION

Selçuk KAYA[1], Hilmi KUSCU[1]*

[1] Trakya University, Engineering Faculty, Mechanical Engineering Department, Edirne, Turkey

Corresponding author e-mail: selcukkaya@trakya.edu.tr

ABSTRACT

The conservation of endemic plants is very important for the diversity of flora and their use in medicine. The fact that some endemic plants are endangered due to various reasons increases this importance even more. For this reason, endemic plants need to be identified and protected. The identification process can sometimes take much longer due to regional conditions. In order to prevent this, an algorithm that detects endemic plants in a region with UAV images will make this process much easier. In order for the algorithm to detect the plants of the region, it must first be trained. Since the training data will be from UAV images, the altitude and camera resolution of the UAV images will be of great importance. Because if the pixel quality is low, the endemic plant will not be able to show its distinctive features with other objects in the region and the plant will not be detected.

Keywords: Endemic Plants, Detection Algortihm, UAV, Camera Resolution, Flight Altitude

INTRODUCTION

Aerial image quality generally depends on two factors, camera resolution and flight altitude. In high altitude such as satellite imagery can cover wider area but will give lower level of detail and a view that can often be blocked by clouds or other obstacles (Rees, 2013). In low altitude such as UAV imagery will give high level of detail and accuracy but may not store a lot of images due to limited card memory. Especially compared to manned fixed-wing aircraft, drones provide lower GSD which is Ground Spacing/Sample Distance (Wallace, Lucieer, Watson & Turner 2012; Watts, Ambrosia & Hinkley, 2012). GSD ratio refers to the size of a pixel in a photo taken from a certain height, so it depends on the flight altitude and the camera resolution. As the flight altitude increases, GSD will also increase but the level of detail will decrease. In order to an algorithm recognize the pattern of the endemic plant in the training process and validation, the edges of the plant parts should be clearly visible and recognizable. More detailed visual data will increase the algorithms detection rate and increase the ability to distinguish from other plants in the area. To achieve this the GSD should be lower as possible. Lowering GSD is possible with using UAV with better camera resolution and taking the UAV images at a low flight altitude. Lowering the flight altitude can increase the time spent since the coverage will be smaller. This will have an impact on the flight duration because of the limitation of battery power.

MATERIAL AND METHOD

The main material for this research will be UAV images of *Centaurea kilaea* in different taken in different flight altitudes and resolution. *Centaurea kilaea* is a perennial endemic plant with morphology of its papi being 3-5 mm. Flowering period is from June to August. It has been found to be effective against breast, cervical, prostate (Şen et al., 2017) and liver cancers

(Şekerler et al., 2020) and has been recommended as a strong candidate for anti-cancer drugs in researches. Currently it is in the EN (Endangered) category on the IUCN Red list due to tourism activities in its habitat.

UAV images were taken by multicopter type UAV named DJI Mini 4 Pro at İğneada Coast. 16 total UAV images of *Centaurea kilaea* were taken in 3, 4, 5, 6, 7, 8, 9 and 10 meter altitudes, 12MP and 48MP resolutions in order to compare the detection rates. The selected algorithm for the research was YOLOv11s, which is a CNN based algorithm. The algorithm was trained with 16 UAV images to analyze the detection rate according to altitude and resolution. Detection rate, detail level and GSD values were compared to see which flight altitude and resolution is the most efficient for algorithm detection and visual detection.

RESULTS AND DISCUSSION

The GSD values for each flight altitude and resolution can be seen in Table 1. As it can seen the image (Figure 1) with 3 meter altitude and 48MP resolution has the lowest GSD which will have the most detail for *Centaurea kilaea*.

Table 1. GSD values for each flight altitude and camera resolution

Flight Altitude/ Resolution	48MP	12MP
3m	0,05	0,10
4m	0,07	0,14
5m	0,09	0,17
6m	0,10	0,21
7m	0,12	0,24
8m	0,14	0,27
9m	0,15	0,31
10m	0,17	0,34

Figure 1. UAV image of dormant *Centaurea kilaea* with lowest GSD value (flight altitude:3m, camera resolution: 48M)

The level of detail can be seen in Figure 2 which has a comparison of zoomed UAV images with 3 meters, 6 meters and 10 meters of altitude in 48MP. Details are very important for distinguishing plants from each other. In 3 meters of altitude, the dormant *Centaurea kilaea* flowers have a shape that can be identified much more easily than other altitudes. When there are similar species in the immediate vicinity of an endemic plant, it helps the observer to distinguish them by providing this detailed information.

Figure 2. Comparison of zoomed UAV images at 48MP (left 3m altitude, middle 6m altitude, right 10 meter altitude)

Algorithm detection rates are shown in Table 2 for each flight altitude and camera resolution. At Figure 3, an example of detection can be seen. Comparing the detection rates the YOLO v11s algorithm which was trained with 16 UAV images of *Centaurea kilaea*, the image taken at 5 meters of altitude and 12MP resolution had the best result. For flight altitudes, images taken at 5 meters, 9 meters and 10 meters performed better than others. For image resolution 48MP had an overall %76,87 success rate and 12MP had %75,75 success rate.

Table 2. Algorithm detection rates for *Centaurea kilaea*

Flight Altitude/ Resolution	**48MP**	**12MP**
3m	%56	%70
4m	%78	%74
5m	%93	%94
6m	%83	%69
7m	%49	%52
8m	%75	%71
9m	%95	%88
10m	%86	%88

Figure 3. Algorithm detected the dormant *Centaurea kilaea* from UAV image taken at 9 meters of altitude and 45MP resolution with %95 percent accuracy.

CONCLUSIONS

Low level of altitudes can provide important details for visual inspection, especially for distinguishing similar species in the same area. However, the UAV flight duration might be an issue according to the battery limitations. This may result in the UAV operator being unable to photograph the entire area in one battery. High altitudes allow you to photograph a larger area in a shorter time. But the trade-off will be the details. Increasing the camera resolution can improve the details while increasing the flight altitude. The observer must find an optimum GSD value that gives the desired details in the UAV image of the endemic plant.

REFERENCES

Şen A., Turan S.A. & Bitiş L. (2017) Bioactivity-guided isolation of anti-proliferative compounds from endemic Centaurea kilaea, *Pharmaceutical Biology*, 55:1, 541-546

Şekerler T, Şen A, Bitiş L, Şener A. (2020) Antihepatocellular carcinoma activity of secondary metabolites of Centaurea kilaea Boiss. *J Res Pharm*.; 24(4): 479-486.

Wallace L., Lucieer A., Watson C., Turner D. (2012). Development of a UAV-LiDAR System with Application to Forest Inventory. *Remote Sens*. 4, 1519–1543.

Watts A.C., Ambrosia V.G., Hinkley E. A. (2012) Unmanned Aircraft Systems in Remote Sensing and Scientific Research: *Classification and Considerations of Use. Remote Sens.* 4, 1671–1692.

Rees, G. (2013). Physical principles of remote sensing. *Cambridge university press.*

APPLICABILTY OF DATA AUGMENTATION TECHNIQUE IN ENDEMIC PLANT IDENTIFICATION ALGORITHMS

Selçuk KAYA[1], Hilmi KUSCU[1]*

[1] Trakya University, Engineering Faculty, Mechanical Engineering Department, Edirne, Turkey

Corresponding author e-mail: selcukkaya@trakya.edu.tr

ABSTRACT

Endemic plants are plants that are distributed in a narrow area and belong only to a certain region. For this reason, they need protection due to their flora diversity and their use in the field of health. Endemic plants can be easily identified and protected by algorithms that can detect them from UAV images. The rarity of most species of endemic plants can make it difficult to create such algorithms. The data set is of great importance in algorithm training. It is only possible for an algorithm to achieve a high level of detection with a large number of data. The rarity of endemic plants will ensure that the image data to be captured will be small, thus making it difficult to train the endemic plant detection algorithm. However, this problem can be solved with various data augmentation methods. In this way, the small number of image data can be increased with data augmentation methods to reach a sufficient number for training.

Keywords: Data Augmentation, Endemic Plant Detection, UAV Imagery, Algorithm Training

INTRODUCTION

Endemism is the state of a species being found only in a single defined geographic location, such as an island, state, nation, country or other defined zone. Endemism is an important concept in conservation biology for measuring biodiversity in a particular place and evaluating the risk of extinction for species. Some of the plants face this risk of extinction very highly and it affects their rareness. When using endemic plant identification algorithms for these rare plants, the data for training will be very low. With the limitation of data, the algorithm cannot recognize the pattern very well which will affect the algorithm accuracy. The experimental results demonstrated that data augmentation-based deep learning approach achieves superior object detection and recognition accuracy compared to traditional training methods without data augmentation (Kaur, Kehehra & Mavi, 2021; Abdulkareem, AL-Shammri, Khalid & Omran, 2024). Data augmentation techniques are used to increase detection accuracy in different areas such as galaxy (Gonzalez, Munoz & Hernandez, 2018), marine organisms (Huang et al., 2019), pedestrian (Liu, Su & Wei, 2022), floating objects in maritime (Mahmoud, Kurniawan, Aneiba & Asyhari, 2024), underwater environments (Noh, Jang, Ha & Park, 2019), railways (Franke, Gopinath, Ristic-Durrant & Michels, 2022), aircraft automatic landing systems safety (Vidimlic, Levin, Loni & Daneshtalab, 2021) and artwork detection (Jeon et al., 2020).

MATERIAL AND METHOD

One individual endemic plant located in the Thrace Region is the main material in this research. This endemic plant is particularly selected due to its rarity rate and endangered status. The endemic plant is *Dianthus ingoldbyi.*

Dianthus ingoldbyi is a perennial plant having petal length 17.48-19.4 mm as morphological data. Flowering period is from June 2nd week to October 3rd week. It is classified as CR (Highly Endangered) according to the IUCN threat category. Since this plant grows on limestone it is heavily threatened by mining activities. The plant can be found in Papaz Beach/Yeniköy, Zunguma Cape/Bozcada, Coast of Dalyan and İbrice Port/Keşan.

Figure 1. Photograph of *Dianthus ingoldbyi* in İbrice Port/Keşan

Figure 2. UAV image of *Dianthus ingoldbyi*

Data augmentation is a technique of artificially increasing the training set by creating modified copies of a dataset using existing data. When collecting images from rare endemic plants, because of the rareness, the data collected will be insufficient for training. In order to increase the amount of image data, image augmentation techniques can be applied. The techniques that will be used are top-down mirroring, left-to-right mirroring, color saturation and random rotation.

The algorithm that will be used is YOLOv8n, a CNN based algorithm that can provide fast and efficient solutions. Algorithms will be trained with two separate datasets which one will be existing dataset and the other one will be augmented dataset. 30 UAV images with different altitudes of *Dianthus ingoldbyi* will be the existing training data and 116 augmented images that was created using augmentation techniques on the existing data will be the augmented training data. After training, two algorithms will be be compared with each other to see which one is more accurate of detecting *Dianthus ingoldbyi*.

RESULTS AND DISCUSSION

As it was mentioned in material and method part 120 augmented images were created using selected augmentation techniques from existing 30 UAV images. 4 of the 120 augmented images were removed from dataset due to similarity. The probability of top-down mirroring was %30, left to right mirroring was %30, color saturation was %20 and random rotation was %30.

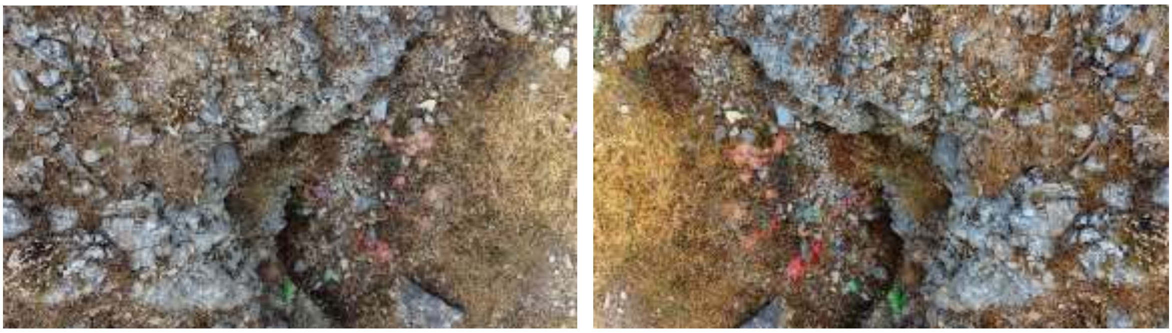


Figure 3. Data augmentation technique (original image on the left, color saturated and left to right mirrored image on the right)

The YOLO algorithm trained with existing data which involved 30 UAV images at 100 epochs. Another same YOLO algorithm was trained with augmented data which involved 116 images at 100 epochs. It has been seen that the first algorithm trained with existing dataset had %67,9 accuracy for detecting *Dianthus ingoldbyi* in easy detection and %27,1 in hard detection.

Class	Images	Instances	Box(P	R	mAP50	mAP50-95)
all	30	52	0.652	0.612	0.679	0.271

Figure 3. Validation of algorithm trained with existing data at Pycharm software

The algorithm that was trained with augmented data had %95,3 accuracy for detecting *Dianthus ingoldbyi* in easy detection and %51,3 in hard detection.

Class	Images	Instances	Box(P	R	mAP50	mAP50-95)
all	116	144	0.892	0.915	0.953	0.513

Figure 4. Validation of algorithm trained with augmented data at Pycharm software

CONCLUSIONS

Data augmentation can increase the detection accuracy for rare objects such as rare and endangered endemic plants which one of them was issued on this study. The algorithms detection accuracy for *Dianthus ingoldbyi* had a %27,4 increase in easy detection and %24 increase in hard detection.

Other image augmentation techniques can be used to see the different results and get more detailed information of which image augmentation techniques can be more efficient. Overfitting can be a serious problem when training an algorithm so removing similar augmented images with a much more precise can avoid overfitting problems.

REFERENCES

Kaur P., Khehra B. S., Mavi E. B. S. (2021). Data Augmentation for Object Detection: A Review, *Midwest Symposium on Circuits and Systems Conference Proceedings*, 537-543

Abdulkareem I. M., AL-Shammri F. K., Khalid, N. A. A., Omran N. A., (2024). A Proposed Approach for Object Detection and Recognition by Deep Learning Models Using Data Augmentation, *International Journal of Online and Biomedical Engineering.* 20(5): 31-43

Gonzalez R. E., Munoz R. P., Hernandez C. A. (2018). Galaxy detection and identification using deep learning and data augmentation, *Astronomy and Computing* 25: 103-109

Huang H., Zhou H., Yang X., Zhang L., Qi L., Zang A. Y. (2019). Faster R-CNN for marine organisms detection and recognition using data augmentation, *Neurocomputing*, 337: 372-384

Mahmoud H., Kurniawan I. F., Aneiba A., Asyhari A.T., (2024). Enhancing Detection of Remotely-Sensed Floating Objects via Data Augmentation for Maritime SAR, *Journal of the Indian Society of Remote Sensing*, 52(6): 1285-1295

Liu B. K., Su S. J., Wei J. Y. (2022). The Effect of Data Augmentation Methods on Pedestrian Object Detection, *Electronics*, 11(19)

Noh J. M., Jang G. R., Ha K. N., Park J. H. (2019). Data Augmentation Method for Object Detection in Underwater Environments, *International Conference on Control Automation and Systems*, 324-328

Franke M., Gopinath V., Ristic-Durrant D., Michels K. (2022). Object-Level Data Augmentation for Deep Learning-Based Obstacle Detection in Railways, *Applied Sciences-Basel*, 12(20)

Vidimlic N., Levin A., Loni M., Daneshtalab M. (2021). Image Synthesisation and Data Augmentation for Safe Object Detection in Aircraft Auto-landing System,*16th International Joint Conference on Computer Vision, Imaging and Computer Graphics Theory and Applications*, 123-135

Jeon H. J., Jung S., Choi Y. S., Kim J. W., Kim J. S. (2020). Object Detection in Artworks Using Data Augmentation, *International Conference on Information and Communication Technology Convergence*, 1312-1314

TECHNICAL AND COST ANALYSIS OF INVERTER SELECTION FOR A FARMHOUSE SOLAR ENERGY SYSTEM

Taşkın Tez[1], Dinçer Akal[2], Ozan Akı[3], İlhan Umut[4]*

[1] Trakya University, Edirne Vocational College of Technical Sciences, Edirne, Turkey
[2] Trakya University, Engineering Faculty, Mechanical Engineering Dept., Edirne, Turkey*
[3] Trakya University İpsala Vocational School, Edirne, Turkey.
[4] Department of Electronics and Automation, Corlu Vocational School, Tekirdağ Namik Kemal University, Tekirdağ, Turkey

Corresponding author e-mail: *dincerakal@trakya.edu.tr*

ABSTRACT

People's energy consumption is increasing significantly as the world's population grows and technology and comfort improve. Fossil resources are still largely used in electricity production. This situation causes the reserves of natural resources to decrease and also causes many environmental problems. For this reason, the transition to renewable energy has gained great importance in the current period and in the future. Direct current produced by photovoltaic panels from solar energy is converted to alternating current with inverters. The choice of inverter to be used in the design for this conversion significantly affects the system efficiency and cost. In this study, the technical and economic analysis of the inverter selection to be used in the solar energy system of a farmhouse located in İskenderköy, close to the center of Edirne, was made in the PV sol application. Upon analysis of the simulation results, it was determined that the initial investment cost for both inverter types in the designed solar energy system is 240.000 TL Furthermore, the payback period of the system was identified as 3.5-3.6 years at an equivalent point in time for both designs.

Keywords: Energy, Photovoltaic Panel, Inverter, Analysis

INTRODUCTION

The widespread use of fossil energy sources in electricity generation brings many problems even in today's conditions. On the other hand, the increase in energy demand and the decrease in fossil resources with the increasing population also increase the interest in cleaner, more environmentally friendly and reliable energy sources (Ikkurti and Saha, 2015, Ekins, 2004). Recent research and developments in renewable energy sources are seen as a source of hope for many countries. In order to meet the energy, need in changing natural conditions, alternative energy sources need to be found and used. Among renewable energy sources, solar energy is known as a sustainable and inexhaustible source with its clean, safe and environmentally friendly features (Ikkurti and Saha, 2015, Goetzberger et al., 2013, Kazmerski, 2006, Nema et al., 2009). Photovoltaic panels are used in converting solar energy into electrical energy. Since photovoltaic panels produce direct current, the obtained energy needs to be converted into alternating current. Inverters are used in solar energy systems for this purpose. Two types of inverters are used in applications. The first of these is the string inverter and the other is the micro inverter. Inverter selection is made according to the power that the solar energy system to be installed will produce. Inverter selection affects the system and its efficiency and investment cost. When the studies on inverter selection in the design of solar energy systems in the literature are examined; System design and micro inverter selection to meet the daily energy needs of low-income households in rural India have been analyzed. It has been confirmed that the use of micro inverter instead of traditional inverter in the proposed design is a more economical solution (Ruchira et al., 2022). In a different study, the total number of active components in photovoltaic systems was reduced and a new microinverter topology was proposed. The simulation results showed that the proposed microinverter provided a higher conversion efficiency (Ternifi et al., 2017). A statistical study carried out in France compared the performance rates of 100 photovoltaic installations using a central inverter and 100 photovoltaic installations using microinverters. According to the data obtained from 200 photovoltaic installations, the performance rate was found to be 79% in both types of inverters. Although the results were almost the same, it was found that microinverters were more preferred due to their lifetime and price, and that the safety of the system in case of fire was better (Lagarde et al., 2023). A resonant high efficiency DC-DC flyback inverter design is proposed for rooftop PV systems. In this study, a 250 W prototype inverter model was implemented and analyzed under different load conditions. According to the results of the study, it was emphasized that the proposed micro-inverter for photovoltaic systems is superior to existing topologies under different weather conditions (Hasan et al., 2017).

In this study, unlike the literature, the technical and economic effects of the inverter type to be selected in the solar energy system to be installed on the roof of a farmhouse in Edirne İskenderköy were analyzed.

MATERIAL AND METHOD

For the 8 kW solar energy system at the current site, 2 different inverter designs were created and simulated using the PV sol application. In the first design, conventional inverters were selected and analyzed, and in the next stage the same analyses were carried out using 5 micro-inverters. All system components are selected and designed in PV sol application and their technical specifications are given in the simulation results.

The configurations of the standard inverter selection in the system installation are given in Table 1.

Table 1. System installation with standard inverter (Configuration 1)

Grid-connected PV System with Electrical Appliances		
Climate Data	Edirne, TUR (2001 - 2020)	
Values source	Meteonorm 8.2(i)	
PV Generator Output	8	kWp
PV Generator Surface	40,0	m²
Number of PV Modules	20	
Number of Inverters	1	
Production Forecast		
PV Generator Output	8,00	kWp
Spec. Annual Yield	1.468,96	kWh/kWp
Performance Ratio (PR)	88,86	%
PV Generator Energy (AC grid)	11.781	kWh/Year
Clipping at Feed-in Point	0	kWh/Year
CO_2 Emissions avoided	5.523	kg / year
Level of Self-sufficiency	40,2	%

PV Generator, 1. Module Area		
Name	Module Area 1	
PV Modules	20 x CWT400-72PM-BC-V (v1)	
Manufacturer	CW Enerji Müh. Tic. ve San. Ltd. Şti.	
Inclination	30	°
Orientation	South 180	°
Installation Type	Roof integrated - rear ventilation	
PV Generator Surface	40,0	m²

Module Area	Module Area 1
Inverter 1	
Model	SUN2000MA-8KTL-M1(High Current version-415Vac) (v1)
Manufacturer	Huawei Technologies
Quantity	1
Sizing Factor	100 %
Configuration	MPP 1: 1 x 10
	MPP 2: 1 x 10

Configurations for micro inverter selection in the system installation are given in Table 2.

Table 2. System installation with micro inverter (Configuration 2)

Grid-connected PV System with Electrical Appliances		
Climate Data	Edirne, TUR (2001 - 2020)	
Values source	Meteonorm 8.2(i)	
PV Generator Output	8	kWp
PV Generator Surface	40,0	m²
Number of PV Modules	20	
Number of Inverters	5	
Production Forecast		
PV Generator Output	8,00	kWp
Spec. Annual Yield	1.476,43	kWh/kWp
Performance Ratio (PR)	89,31	%
PV Generator Energy (AC grid)	11.834	kWh/Year
Clipping at Feed-in Point	0	kWh/Year
CO_2 Emissions avoided	5.551	kg / year
Level of Self-sufficiency	40,1	%

PV Generator, 2. Module Area

Name	Module Area 2	
PV Modules	20 x CWT400-72PM-BC-V (v1)	
Manufacturer	CW Enerji Müh. Tic. ve San. Ltd. Şti.	
Inclination	30	°
Orientation	South 180	°
Installation Type	Roof integrated - rear ventilation	
PV Generator Surface	40,0	m²

Module Area	Module Area 2
Inverter 1	
Manufacturer / Model	Deye / Micro Inverter SUN 1600 (v3)
Quantity / Sizing Factor	1 / 100 %
Configuration	MPP 1:1x1, MPP 2:1x1, MPP 3:1x1, MPP 4:1x1
Inverter 2	
Manufacturer / Model	Deye / Micro Inverter SUN 1600 (v3)
Quantity / Sizing Factor	1 / 100 %
Configuration	MPP 1:1x1, MPP 2:1x1, MPP 3:1x1, MPP 4:1x1
Inverter 3	
Manufacturer / Model	Deye / Micro Inverter SUN 1600 (v3)
Quantity /	1 / 100 %
Configuration	MPP 1:1x1, MPP 2:1x1, MPP 3:1x1, MPP 4:1x1
Inverter 4	
Manufacturer / Model	Deye / Micro Inverter SUN 1600 (v3)
Quantity / Sizing Factor	1 / 100 %
Configuration	MPP 1:1x1, MPP 2:1x1, MPP 3:1x1, MPP 4:1x1
Inverter 5	
Manufacturer / Model	Deye / Micro Inverter SUN 1600 (v3)
Quantity / Sizing Factor	1 / 100 %
Configuration	MPP 1:1x1, MPP 2:1x1, MPP 3:1x1, MPP 4:1x1

RESULTS AND DISCUSSION

Simulation results are evaluated according to the type of inverter to be used in the designed solar energy system. Similar results are obtained from technical and economic analyses in both inverter types with the same investment cost.

The simulation results obtained when a standard inverter is used in the system are given in Table 3.

Table 3. Simulation results obtained by using standard inverter

Total investment costs	240.000,00	₺
Internal Rate of Return (IRR)	76,92	%
Amortization Period	3,6	Years
Electricity Production Costs	1,1317	₺/kWh
Energy Balance/Feed-in Concept	Net-Metering	
PV System		
PV Generator Output	8,00	kWp
Spec. Annual Yield	1.468,96	kWh/kWp
Performance Ratio (PR)	88,86	%
PV Generator Energy (AC grid)	11.781	kWh/Year
Clipping at Feed-in Point	0	kWh/Year
CO_2 Emissions avoided	5.523	kg / year
Appliances		
Appliances	7.570	kWh/Year
Standby Consumption (Inverter)	29	kWh/Year
Total Consumption	7.599	kWh/Year
Power Surplus	4.181,7	kWh
Solar Fraction	155,0	%
Level of Self-sufficiency		
Total Consumption	7.599	kWh/Year
covered by grid	4.545	kWh/Year
Level of Self-sufficiency	40,2	%
System Data		
PV Generator Energy (AC grid)	11.781	kWh/Year
PV Generator Output	8	kWp
Start of Operation of the System	18.01.2024	
Assessment Period	20	Years
Interest on Capital	1	%
Economic Parameters		
Internal Rate of Return (IRR)	76,92	%
Accrued Cash Flow (Cash Balance)	1.600.925.512,42	₺
Amortization Period	3,6	Years
Electricity Production Costs	1,1317	₺/kWh
Payment Overview		
Specific Investment Costs	30.000,00	₺/kWp
Investment Costs	240.000,00	₺
One-off Payments	0,00	₺
Incoming Subsidies	0,00	₺
Annual Costs	0,00	₺/Year
Other Revenue or Savings	0,00	₺/Year

Remuneration and Savings	
Total Payment from Utility in First Year	13.067,59 ₺/Year
First year savings	15.073,04 ₺/Year
tedas_2 (Example)	
Energy Price	1,9912 ₺/kWh
Compensation for Surplus	3,1249 ₺/kWh
Inflation Rate for Energy Price	71.6 %/Year

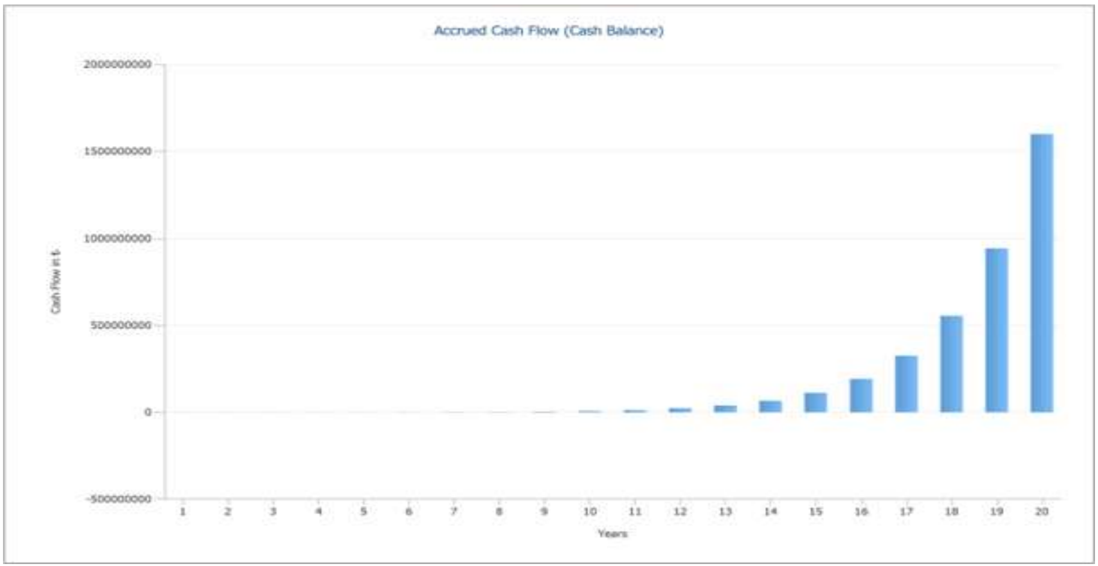


Figure1. Accumulated Cash Flow for Configuration 1

The cash flow graph of the system designed with a conventional inverter is shown in Figure 1. Analyzing the graph, it can be seen that the system pays back the investment cost in 3.6 years. The simulation results obtained when a microinverter is used in the system are given in Table 4.
The cash flow graph of the microinverter system is shown in Figure 2. Looking at the graph, we can see that the investment cost of the system is paid back in the same period, 3.6 years.

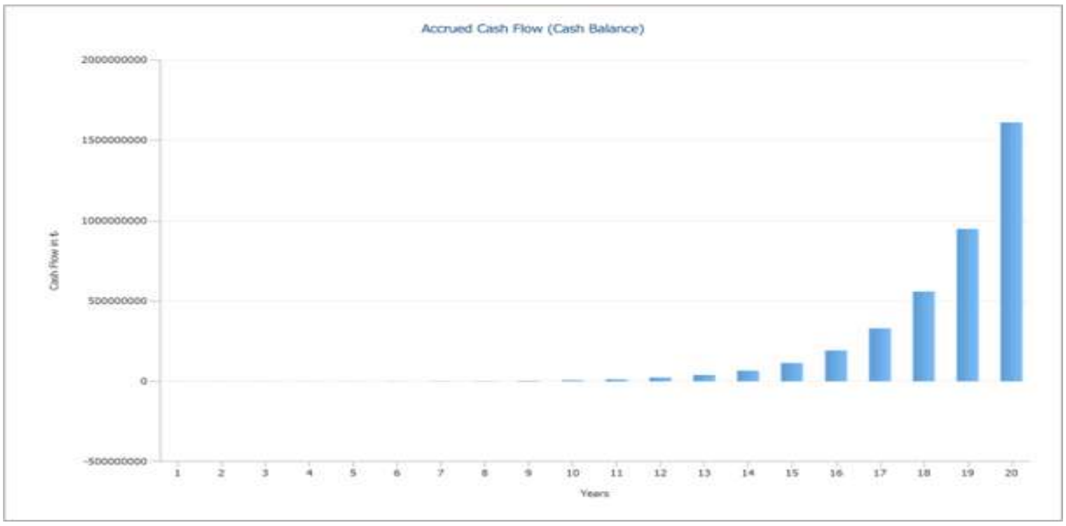


Figure 2. Accumulated Cash Flow for Configuration 2

Table 4. Simulation results obtained using micro inverter

Your Gain		
Total investment costs	240.000,00	₺
Internal Rate of Return (IRR)	77,04	%
Amortization Period	3,5	Years
Electricity Production Costs	1,126	₺/kWh
Energy Balance/Feed-in Concept	Net-Metering	
PV System		
PV Generator Output	8,00	kWp
Spec. Annual Yield	1.476,43	kWh/kWp
Performance Ratio (PR)	89,31	%
PV Generator Energy (AC grid)	11.834	kWh/Year
Clipping at Feed-in Point	0	kWh/Year
CO_2 Emissions avoided	5.551	kg / year
Appliances		
Appliances	7.570	kWh/Year
Standby Consumption (Inverter)	23	kWh/Year
Total Consumption	7.593	kWh/Year
Power Surplus	4.241,4	kWh
Solar Fraction	155,9	%
Level of Self-sufficiency		
Total Consumption	7.593	kWh/Year
covered by grid	4.548	kWh/Year
Level of Self-sufficiency	40,1	%
System Data		
PV Generator Energy (AC grid)	11.834	kWh/Year
PV Generator Output	8	kWp
Start of Operation of the System	18.01.2024	
Assessment Period	20	Years
Interest on Capital	1	%
Economic Parameters		
Internal Rate of Return (IRR)	77,04	%
Accrued Cash Flow (Cash Balance)	1.611.539.453,20	₺
Amortization Period	3,6	Years
Electricity Production Costs	1,126	₺/kWh
Payment Overview		
Specific Investment Costs	30.000,00	₺/kWp
Investment Costs	240.000,00	₺
One-off Payments	0,00	₺
Incoming Subsidies	0,00	₺
Annual Costs	0,00	₺/Year
Other Revenue or Savings	0,00	₺/Year

Remuneration and Savings	
Total Payment from Utility in First Year	13.254,13 ₺/Year
First year savings	15.073,04 ₺/Year
tedas_2 (Example)	
Energy Price	1,9912 ₺/kWh
Compensation for Surplus	3,1249 ₺/kWh
Inflation Rate for Energy Price	71.6 %/Year

CONCLUSIONS

In this study, the effect of the inverter type to be used in the solar energy system to be installed in a small-scale individual farmhouse is analyzed with PV sol application. The investment cost for each inverter type is determined as 240 000 TL. When the data obtained from the simulation results are evaluated, it is seen that the investment cost for both inverter types is almost the same for 3.5-3.6 years. However, it is observed that 53 kWh more electricity is generated every year in the design using micro inverter. Therefore, when the system is operated under real conditions, it is understood that the use of micro inverter will be more advantageous in terms of performance and failure.

REFERENCES

Ekins, P. 2004. Step changes for decarbonizing the energy system: research needs for renewables, energy efficiency and nuclear power. Energy Policy, 32, 17, 1891-1904.

Goetzberger, A., Hebling, C., Schock, H-W. 2013. Photovoltaic materials, history, status and Outlook. Materials Science and Engineering, R, 40, 1-46.

Hasan,R., Mekhilef, S., Seyedmahmoudian, M., Horan, B. 2017. Grid-connected isolated PV microinverters: A review. Renewable and Sustainable Energy Reviews, 67, 1065-1080.

Ikkurti, P., Saha, S. 2015. A comprehensive techno-economic review of microinverters for Building Integrated Photovoltaics (BIPV). Renewable and Sustainable Energy Reviews, 47, 997-1006.

Kazmerski, L.L, 2006. Solar photovoltaics R&D at the tipping point: A 2005 technology overview. Journal of Electron Spectroscopy and Related Phenomena, 150, 105-135.

Lagarde, Q., Beillard, B., Mazen, S., Denis, M. Leylavergne, J. 2023. Performance ratio of photovoltaic installations in France: Comparison between inverters and micro-inverters. Journal of King Saud University– Engineering Sciences, 35, 531-538.

Nema, P., Nema, K.R.,Rangnekar, S. 2009. A current and future state of art development of hybrid energy system using wind and PV-solar: A review. Renewable and Sustainable Energy Reviews. 13, 2096-2103.

Ruchira, R., Tamrakar, E., Patel, N.R., Choudekar, P. 2022. Customized design of microinverter based solar photovoltaic system for small houses in developing nations. Renewable Energy Focus,42, 178-189.

Ternifi, T.E.Z., Petit, P., Bachir,G., Aillerie, M. 2017. New Topology of Photovoltaic Microinverter based on Boost converter. Energy Procedia, 119, 938-944.

EFFECT OF TILT ANGLE OF PHOTOVOLTAIC PANELS ON POWER OUTPUT WHEN INSTALLING SOLAR ENERGY SYSTEMS ON RESIDENTIAL BALCONY WALLS

Dinçer Akal[1], Ozan Akı[2], Taşkın Tez[3], İlhan Umut[4]*

[1] Trakya University, Engineering Faculty, Mechanical Engineering Department, Edirne, Turkey*
[2] Trakya University Ipsala Vocational School, Edirne, Turkey.
[3] Trakya University, Edirne Vocational College of Technical Sciences, Edirne, Turkey
[4] Department of Electronics and Automation, Corlu Vocational School, Tekirdag Namik Kemal University, Tekirdag, Turkey

Corresponding author e-mail: *dincerakal@trakya.edu.tr*

ABSTRACT

Our country is in an advantageous position compared to many other countries in terms of solar energy use. However, solar energy systems require a significant investment budget in terms of initial installation costs. Due to the lack of incentives for rooftop installations in some countries, small-scale solar energy conversion systems on individual balconies are promising. However, the amount of energy produced by balcony solar energy systems varies depending on the tilt angle of the photovoltaic panels. In this study, the effect of the tilt angle of a 100W photovoltaic panel mounted on the facade wall of a solar energy conversion system installed on the campus of Trakya University Faculty of Engineering was experimentally investigated for one month. When the results were analyzed, the instantaneous maximum power output of the photovoltaic panel was measured as 25W when the inclination angle was 90° even on the day with the highest solar radiation, while this value was measured as 65W when the inclination angle was 30°. It was found that energy efficiency decreased significantly when a 90° photovoltaic panel was applied to the facade or balcony wall. For this reason, it is necessary to specify the tilt angle of the photovoltaic panels in balcony solar energy system installations.

Keywords: Energy, Photovoltaic panel, balcony wall, tilt angle

INTRODUCTION

Recently, solar power has become one of the most popular renewable energy sources in many countries. Our country is in a more advantageous position than many countries due to its location and climate characteristics. In addition, the installed power of a system designed for a specific location and the amount of energy that can be produced according to climate characteristics can be analyzed quite easily with the simulation programs developed today. When people think of solar energy systems, they usually think of solar power plants and rooftop photovoltaic panels.

In today's conditions, solar energy systems have started to be used not only on roofs, but also in more accessible areas such as terraces, balconies, car parks and facade systems. In addition to the use of existing roof areas, building-integrated photovoltaic panel applications offer the opportunity to benefit from some advantages (Skandalos and Karamanis, 2021; Aguacil et al., 2019). Especially in high-rise buildings, where the roof area covered by photovoltaic panels is limited, the usability of the facade area should be evaluated (Tian et al., 2023). With this in mind, the application of photovoltaic panels on the facades of high-rise buildings in China has been commercialized and its use has become widespread. In addition, photovoltaic panels with different characteristics are produced to meet the needs of building façade applications (Aguacil et al., 2019). The use of building integrated photovoltaic panels also reduces greenhouse gas emissions by reducing the use of fossil fuels (Aguacil et al., 2024). In addition, cantilevered balconies, balcony railings and building shading also offer benefits for photovoltaic panel applications. These applications need to be promoted, and architectural studies are needed (Xiang et al., 2023). The integration of photovoltaic panels into the building, especially in adjacent apartments, and the presence of air conditioning units on the external facades pose some installation difficulties. In these cases, architectural solutions and building-specific designs are required. When transparent and semi-transparent photovoltaic panels are used in building components such as windows and balcony railings, they provide lighting as well as electricity. Despite all this, there are still incorrect applications that reduce the energy efficiency of photovoltaic panel installations. The most common mistakes are the failure to carry out technical and economic analyses, the incorrect selection of system components in the design and the incorrect tilt angle of the photovoltaic panels. It should be remembered that the maximum conversion efficiency of photovoltaic panels can be obtained by avoiding these most common errors. To support the use of building-integrated photovoltaic systems in Scandinavian countries and to estimate the solar energy potential, solar radiation values from balconies, external staircases and between buildings have been analyzed (Xiang et al., 2023). A similar approach has been used in Beijing to conduct extensive research on the renovation and optimization of balconies in old residential buildings. The characteristics and models of residential balconies were simulated and new designs were optimized (Li et al., 2023). A simulation case study found that although the roofs of buildings are generally suitable for photovoltaic panels, energy losses occur due to shading, especially in large cities and high-rise buildings, and in such cases photovoltaic panels installed on the south facades of buildings give better results (Vulkan et al., 2018).

In this study, unlike the literature on solar electricity generation, the effect of a photovoltaic panel mounted on the balcony railing of a house with a 90° vertical and 30° inclination angle on energy efficiency was experimentally investigated.

MATERIAL AND METHOD

The installation shown in Figure 1 was carried out in order to investigate the effect of the inclination angle given to the photovoltaic panels on the energy production in the balcony applications on the south facade of the Trakya University Faculty of Engineering campus. Photovoltaic panels with the same characteristics and equivalent output power of 100W were used in this experimental study. As seen in Figure 1, one of the photovoltaic panels was mounted on the balcony railing at a 90° vertical angle (PV panel B) and the other PV panel C was mounted on the balcony railing at a 30° inclination. Then, the power output cables from both PV panels were connected to the data logger. The power outputs of the PV panels were measured every 15 minutes for 30 days starting from June 01, 2024, and recorded on the system's memory card. At the end of the experiments, a random day and the best day in June were selected and the energy value graphs obtained from the PV panels were drawn.

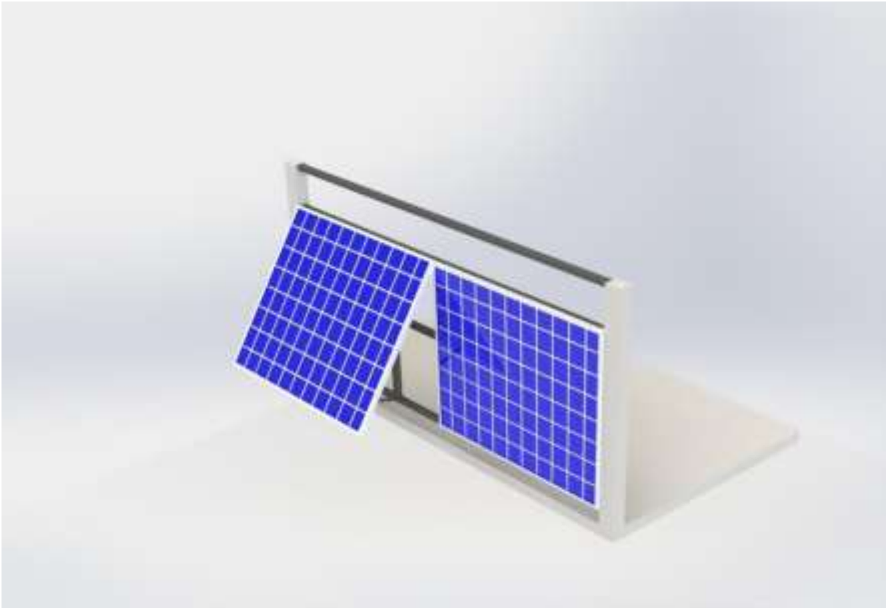


Figure 1. Installation of PV Panels in Balcony Application

RESULTS AND DISCUSSION

At the end of the study, the total energy production was calculated as a function of the voltage and current values obtained from the photovoltaic panels. It was observed that the power output of the photovoltaic panel applied at a 30° angle was higher on all days in June. It was also observed that the total amount of energy obtained from the photovoltaic panel applied at a vertical angle of 90° (PV panel C) was much lower than that of PV panel B applied at an angle of 30°.

Figure 2 shows the power output of the photovoltaic panel mounted on the balcony railing at a tilt angle of 30°. When analyzing the data in June, the maximum power output was around 65W. This power value was obtained on 16 June 2024.

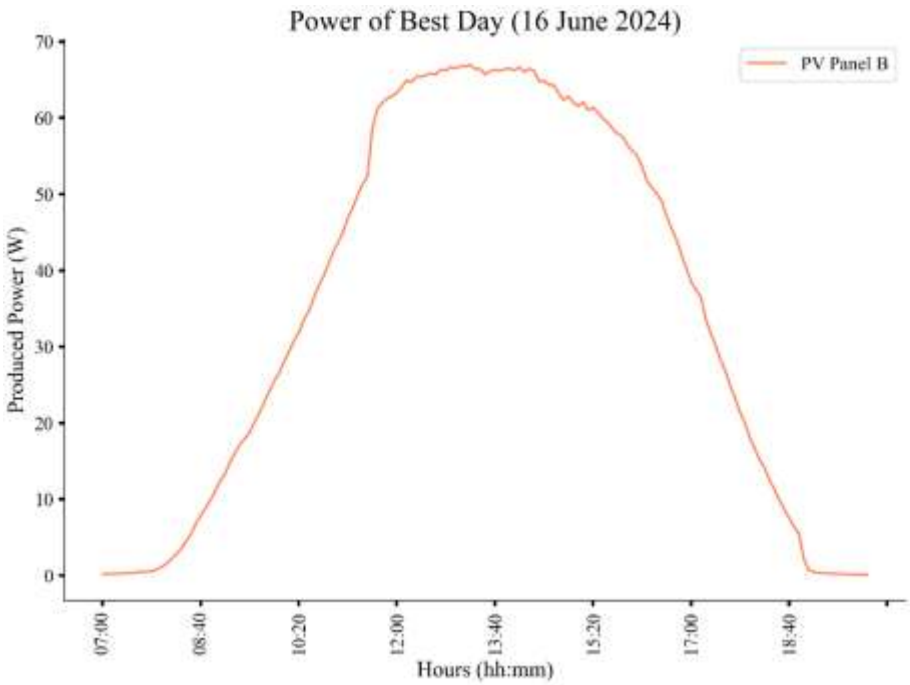


Figure 2. The day when the PV Panel B achieves the highest power output

A random day in June (June 9, 2024) was chosen and the power output from the PV panels was analyzed. The output power levels of the PV panel on the randomly selected day are shown in Figure 3. It was observed that a maximum power output of 60W was obtained from PV Panel B, which was applied at a tilt angle of 30° on a randomly selected day.

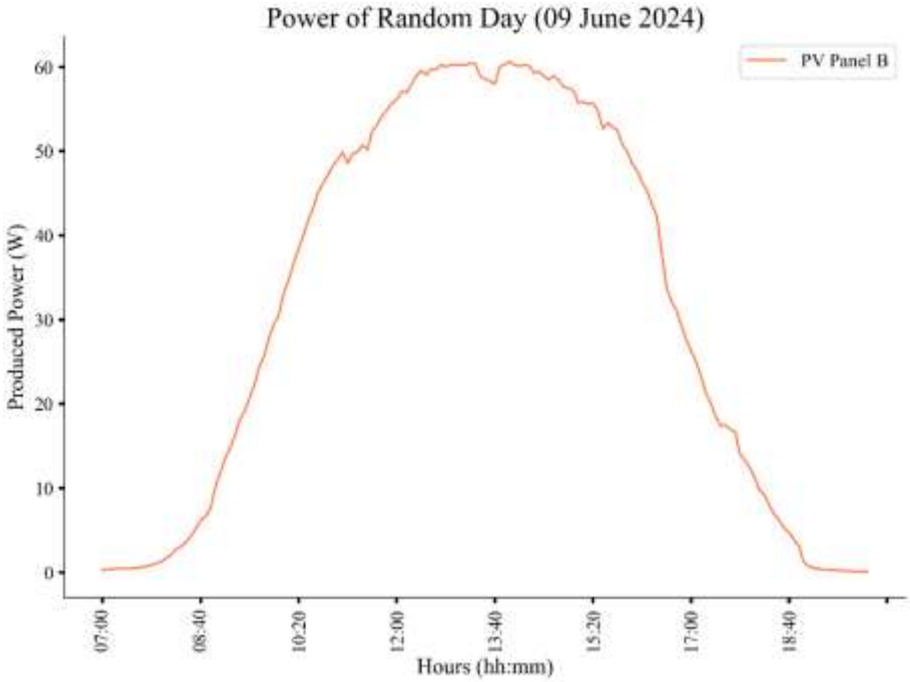


Figure 3. PV Panel B power output on a random day

Figure 4 shows the power output of the photovoltaic panel mounted vertically at 90° to the balcony railing. When the data in June was analyzed, the maximum power output was about 25W. This power value was obtained on June 16, 2024.

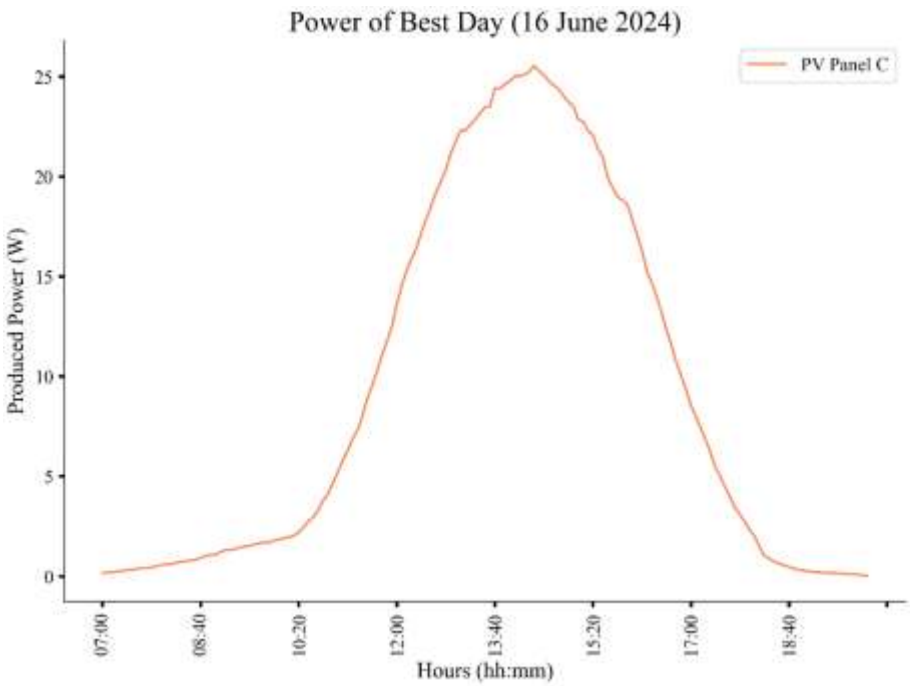


Figure 4. The day when the PV Panel C achieves the highest power output

A random day in June (June 9, 2024) was selected and the power output from the PV panels was analyzed. The output power levels of the PV panel on the randomly selected day are shown in Figure 5. It was observed that a maximum power output of 6.3W was obtained from PV Panel C applied at 90° vertical on a randomly selected day.

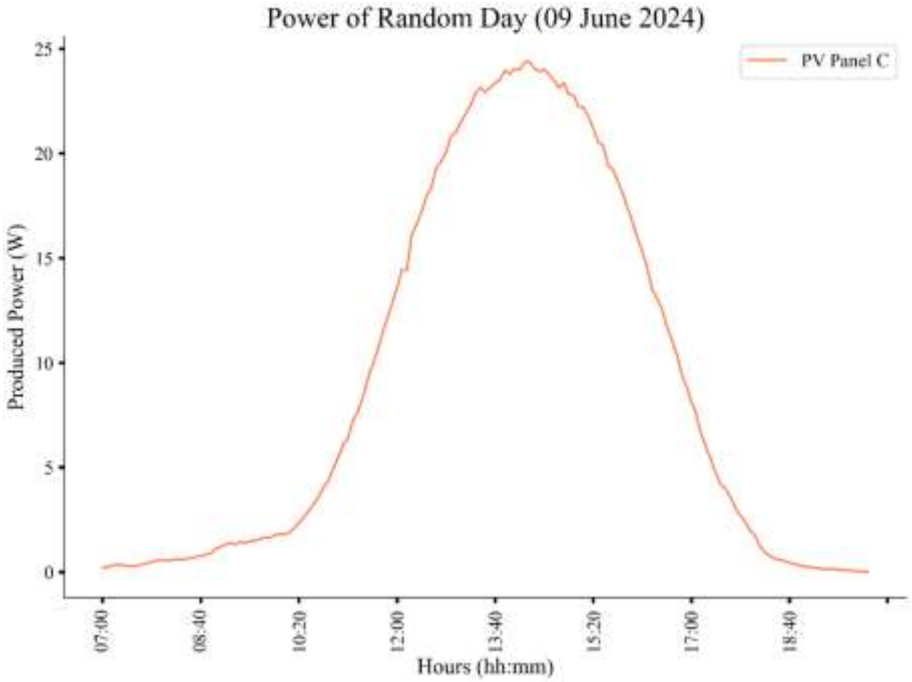


Figure 5. PV Panel B power output on a random day

It can be seen that when the photovoltaic panel is applied vertically at 90° (PV panel C), the power output is at a very low level compared to PV panel B with an inclination angle of 30°. At the end of the study, the total amount of energy obtained from the PV panels was calculated and given graphically in Figure 6. When Figure 6 is examined, it is seen that when the photovoltaic panel (PV Panel B) is given a 30° inclination angle, it produces twice as much energy as the photovoltaic panel (PV Panel C) applied vertically at 90°.

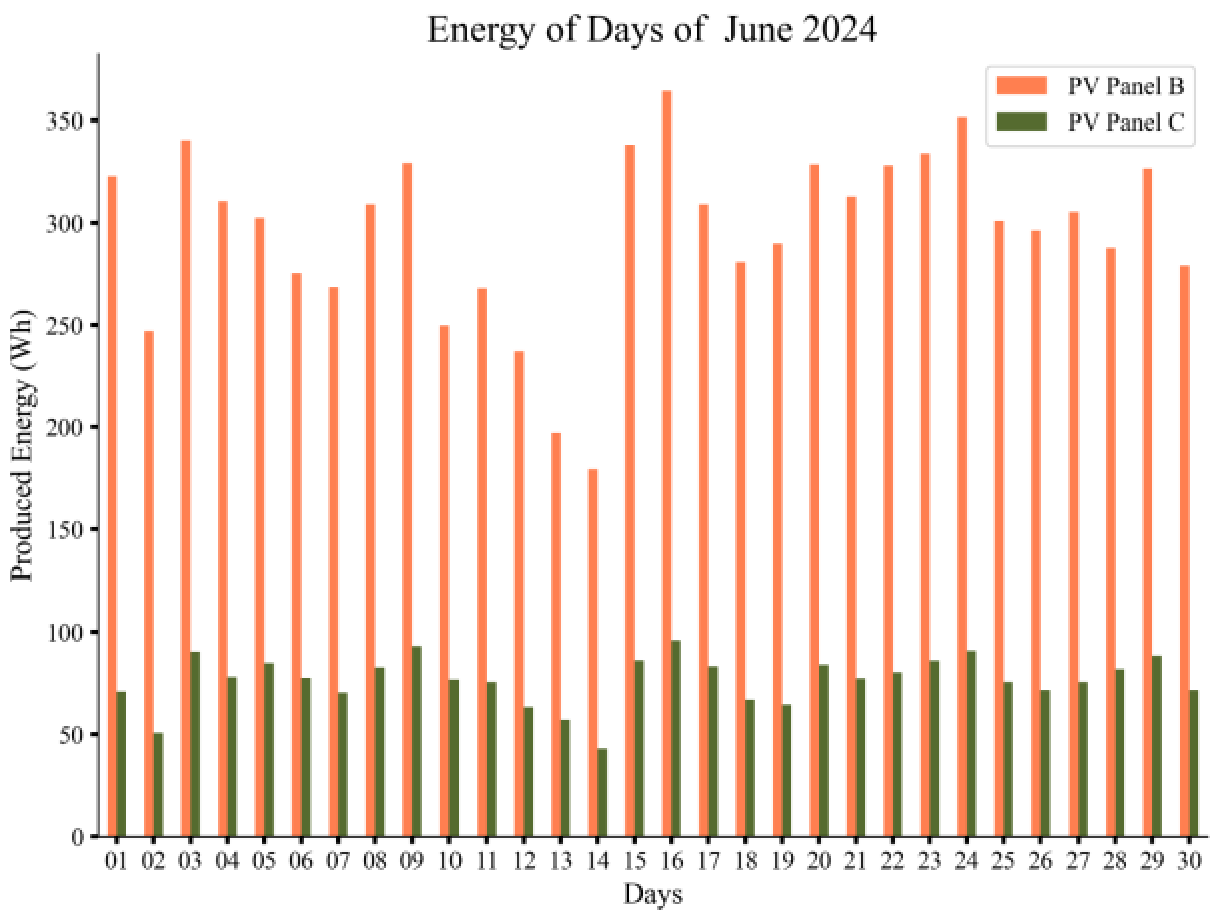


Figure 6. Monthly total energy levels produced in PV panel B and PV panel C

When Figure 6 is examined, it is seen that the photovoltaic panel (PV Panel C) applied at a 90° vertical angle could not even reach the 100Wh level in daily energy production in June.

CONCLUSIONS

In this study, the effect of the tilt angle of photovoltaic panels mounted on the balcony wall on the power output of a solar energy system installed in the campus of Trakya University Faculty of Engineering was investigated. The effect of the tilt angle of 100W monocrystalline photovoltaic panels on the power output and energy production in the designed system was analyzed. According to the results obtained from the experiments, even on the day when the solar radiation was at its highest, the instantaneous maximum power output was measured to be 25W when the tilt angle of the photovoltaic panel was 90°, and this value was determined to be 65W when the tilt angle was 30°. It was found that the energy efficiency decreased significantly when 90° photovoltaic panels were mounted on the facade or balcony wall. Therefore, when installing solar energy systems, the tilt angle of the photovoltaic panels should be determined according to the geographical location of the dwellings and the architectural features of the balcony/facade.

REFERENCES

Aguacil, S., Duque, S., S. Lufkin, S., Rey, E. 2024. Designing with building-integrated photovoltaics (BIPV): A pathway to decarbonize residential buildings. Journal of Building Engineering, 96, 110486.

Aguacil, S., Lufkin, S., Rey, E. 2019. Active surfaces selection method for building-integrated photovoltaics (BIPV) in renovation projects based on self-consumption and self-sufficiency. Energy & Buildings, 193, 15-28.

Li, N., Miao, X., Geng, W., Li, Z., Li, L. 2023. Comprehensive renovation and optimization design of balconies in old residential buildings in Beijing: A study. Energy & Buildings, 295, 113296.

Skandalos, N., Karamanis, D. 2021. An optimization approach to photovoltaic building integration towards low energy buildings in different climate zones. Applied Energy, 205, 117017.

Tian, X., Wang J., Ji, J. 2023. Performance prediction of a curved-type solar balcony combined with the flexible PV/T system during the non-heating season. Energy Conversion and Management, 292, 117402.

Vulkan, A., Kloog, I., Dorman, M., Erell, E. 2018. Modeling the potential for PV installation in residential buildings in dense urban areas. Energy & Buildings, 169, 97-109.

Xiang, C., Matusiak, S. B. 2023. Façade Integrated Photovoltaics design for high-rise buildings with balconies, balancing daylight, aesthetic and energy productivity performance. Journal of Building Engineering, 57, 104950.

ELECTRICITY PRODUCTION AND COST ANALYSIS USING SOLAR PANELS ON AGRICULTURAL FENCES

Dinçer Akal[1] , Taşkın Tez[2]*

[1*]*Trakya University, Engineering Faculty, Mechanical Engineering Department, Edirne, Turkey*

[2] *Edirne Provincial Directorate of Health, Ministry of Health, Edirne, Turkey.*

Corresponding author e-mail: dincerakal@trakya.edu.tr

ABSTRACT

With the gradual depletion of fossil fuel resources, costs and environmental problems, the use of renewable energy is becoming more important every day. Recently, there has been a growing interest in solar energy as one of the renewable energy sources. However, problems still occur in solar power plants due to incorrect design and application in operating conditions. In particular, when mistakes are made in the selection of system components and in the economic analysis, both the investment cost and the payback period are adversely affected. In this study, the aim is to produce electrical energy and cost analysis by using photovoltaic panels in agricultural fences to be used at the borders of land in Edirne central location for site selection in a solar power plant. The economic analysis of the photovoltaic panel fence system designed for a 100 kWh solar power plant at the determined site was carried out using the PV sol program. According to the simulation results obtained, the investment cost of the designed system was found to be 3 278 880TL and the payback period was 7.1 years.
Keywords: Energy, economic analysis, solar panel, fence.

INTRODUCTION

Recently, the environmental damage caused by the global use of fossil fuels, the depletion of resources and increasing global warming have accelerated the interest in renewable energy sources. In this context, if we look at the types of renewable energy sources, we can see that solar energy installations have increased significantly. Considering its geographical location and annual sunshine hours, Turkey has a higher solar energy potential than many other countries. In addition, the conversion efficiency of solar energy varies depending on the installation conditions. The climatic conditions of the installation region, the correct angle and orientation of the solar panels, the selection of the inverter and the correct selection of other system components are of great importance. Therefore, before installing solar power plants in any location, the production and amortisation periods must be analysed using simulation programs. Important parameters to evaluate are efficiency, performance ratio and system losses. The performance ratio varies depending on the environmental conditions, installation characteristics and design (Kumar el al, 2021; Nwaigwe et al, 2019; Sarraf et al, 2016; Vaka and Talukdar, 2020).[1-2-3-4]
As a result of the evaluation of all these parameters, wrong applications in the installation of solar power plants will be prevented. Many different simulation programmes are used for the installation and analysis of solar power plants. When some studies in the literature on this subject are examined; PVsyst simulation program was used for PV system installation and loss analysis at Bikaner University in India. While the annual average energy requirement was 1086.24 kWh for the office in mechanical engineering, the energy obtained from the solar panel was determined as 1143.6 kWh. At the end of the analysis, it was found that different power

sources caused losses in the system and the annual average performance rate was found to be 72%(Kumar el al, 2021). There are some difficulties in selecting the simulation programs to be used in applications. In order to review the existing programs, four system-based (System Advisor Model (SAM), PVsyst, PV*Sol and Solarius PV) and three online software tools (Helioscope, PV-Watts and PV-GIS) were examined under seven categories. It was observed that the least errors occurred in the data recorded with PV*Sol and Solarius PV (Kaleshwarwar and Bahadure, 2023). In a study conducted in Bangladesh, a comprehensive technical and economic analysis was conducted for the configuration of PV modules to benefit from solar energy in the best conditions. For this purpose, the effects of different solar modules such as monofacial, bifacial, dual-axis solar tracker and seasonally inclined solar module were examined. Similarly, in this study, energy efficiency in Bangladesh was evaluated using PV*SOL, PVsyst and System Advisor Model (SAM) software. According to the results obtained from this study, the highest annual energy production was achieved with the dual-axis solar tracker system, but it was stated that there may be difficulties in implementation due to the higher investment cost. It was observed that the simulation programs used in this study have great importance in terms of energy efficiency and economic analysis (Oyshei et al, 2024). In a study where comparative economic analyses of residential solar photovoltaic systems in Canadian provinces were conducted, investment cost, payback period and efficiency concepts were evaluated using data from 2013 and 2016. The results were presented in summary tables showing selected major cities classified into different categories, classified according to financial viability. In this study, sensitivity analyses were conducted to determine new values for electricity sales price, investment cost of the proposed project and required subsidy in order to make the project attractive for a short payback period of 5 years for residential buildings (Sow et al, 2019). In another study, the University of Jordan conducted a technical and economic analysis of a grid-connected solar energy system for the transition to renewable energy due to high electricity consumption. After evaluating parameters such as performance analysis, economic analysis, efficiency and land use, it was found that the payback period was 3 years with a return of 32% (Ayadi et al, 2018). Techno-economic analysis was performed using RETScreen simulation program for a 50 MW solar energy system in a selected field in Afghanistan. Based on this study data, it was stated that the highest annual energy production potential (166336 MWh/year) could be obtained with the dual-axis tracking system (Hamad et al, 2024).

In this study, unlike the literature, in case of using PV Panels instead of normal fences on the borders of a selected site in Edirne, electrical energy production, investment cost and depreciation period of the system were analyzed.

MATERIAL AND METHOD

It was aimed to generate electricity from solar energy by applying photovoltaic panels instead of normal steel wire fence material in determining the boundaries of the field whose location is shown in Figure 1 in the center of Edirne province. In the fence design, 144 CW brand CWT690-132TNB12 Bifacial Monocrystalline PV Panels were selected, 90° angle was applied, inverter and other system components were selected and modelling was done. Using the current location information for the design, investment cost, electrical energy production amount and depreciation period were analysed for grid connected self-consumption solar energy conversion system in PV sol simulation program.

Figure 1. PV panel application location

An example of a fence to be built from solar panels to be applied to the land border area is shown in Figure 2. Thus, electrical energy will be produced with solar panels by utilising the area on the border line.

Figure 2. Application model of a fence made of solar panels (www.next2sun.com).

All data and technical details included in the system installation are explained under detailed headings in Table 1.

Table 1: Configurations for system installation

1.1.Climate Data

Location	Edirne , TUR (2001 - 2020)
Values source	Meteonorm 8.2(i)
Resolution of the data	1 h
Simulation models used:	
- Diffuse Irradiation onto Horizontal Plane	Hofmann
- Irradiance onto tilted surface	Hay & Davies

1.2. Module Area (PV Generator, 1. Module Area)

Name	
PV Modules	144 x CWT690-132TNB12 (v1)
Manufacturer	CW Enerji Müh. Ticaret ve San. Ltd. Şti.
Inclination	90°
Orientation	South 200°
Installation Type	Mounted - Open Space
PV Generator Surface	447,3 m^2

1.3.Configuration

Module Area	Modül Alanı 1
Inverter 1	
Model	SUN2000-40KTL-M3（480Vac） (v2)
Manufacturer	Huawei Technologies
Quantity	2
Sizing Factor	124,2 %
Configuration	MPP 1: 1 x 18
	MPP 2: 1 x 18
	MPP 3: 1 x 18
	MPP 4: 1 x 18

1.4.Production Forecast

PV Generator Output	99,36	kWp
Spec. Annual Yield	957,46	kWh/kWp
Performance Ratio (PR)	62,13	%
Grid Export	95.182	kWh/Year
Grid Export in the first year (incl. module degradation)	95.182	kWh/Year
Standby Consumption (Inverter)	48	kWh/Year
CO_2 Emissions avoided	44.713	kg / year

RESULTS AND DISCUSSION

In this study, the simulation results obtained when the data belonging to the design system components were evaluated are explained in detail in Table 2.

Table 2: Simulation results

2.1.System Data		
Grid Export in the first year (incl. module degradation)	95.182	kWh/Year
PV Generator Output	99,4	kWp
Start of Operation of the System	1.07.2024	
Assessment Period	20	Years
Interest on Capital	1	%

2.2.Economic Parameters		
Internal Rate of Return (IRR)	65,21	%
Accrued Cash Flow (Cash Balance)	22.533.883.646,67	₺
Amortization Period	7,1	Years
Electricity Production Costs	1,91	₺/kWh

2.3.Payment Overview		
Specific Investment Costs	33.000,00	₺/kWp
Investment Costs	3.278.880,00	₺
One-off Payments	0,00	₺
Incoming Subsidies	0,00	₺
Annual Costs	0,00	₺/Year
Other Revenue or Savings	0,00	₺/Year

2.4.Remuneration and Savings		
Total Payment from Utility in First Year	24.909,05	₺/Year
Validity	28.07.2024 -	27.07.2044
Specific feed-in / export Remuneration	0,1873	₺/kWh
Feed-in / Export Tariff	24909,0545	₺/Year
Inflation Rate for Feed-in	71,60	%/Year

The electric energy obtained according to the months through the designed system is given in Figure 3.

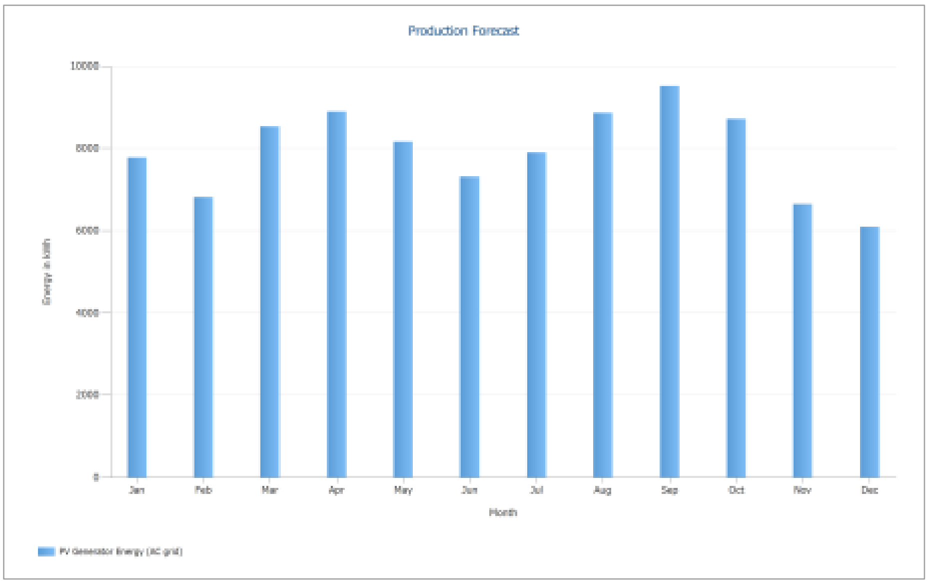


Figure 3. Total electrical energy levels generated by month

The graph showing the amortisation period of the designed solar fence system is given in Figure 4. When Figure 4 is analysed, it is seen that the cash flow and amortisation period obtained from the system is 7.1 years.

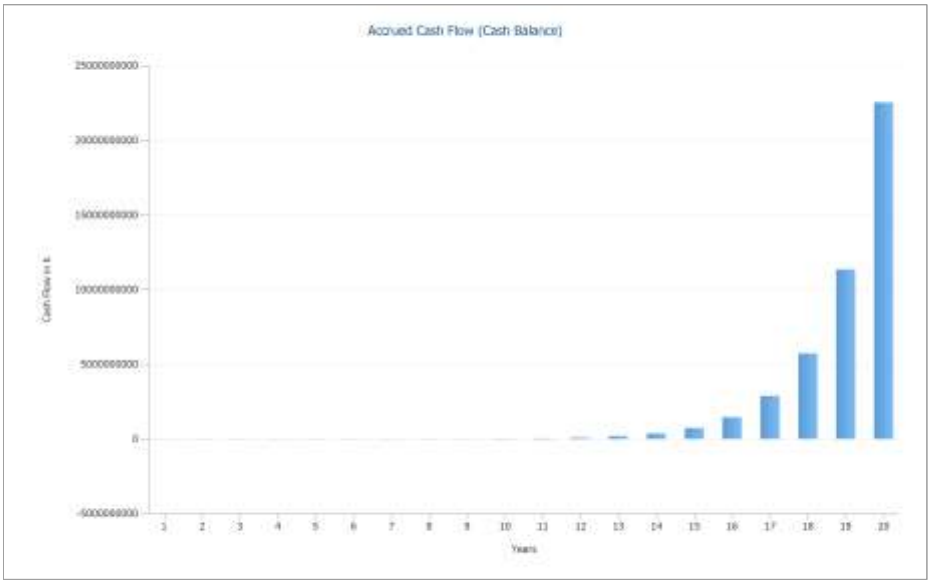


Figure 4. Accumulated Cash Flow

CONCLUSION

In the study, the investment cost and the depreciation period according to the cash flow obtained with the solar fence application in determining the boundaries of a land in the central location of Edirne were analyzed. When the PV Sol simulation results were evaluated; the total investment cost was found to be 3278880 TL the internal rate of return was 65.21%, the electricity production cost was 1.91TL and the amortization period was 7.1 years. Another important result is that the 447.3 m² area required for 100 kW electricity generation in the solar energy conversion plant will be obtained by the photovoltaic panel fence application designed without the need for any land. Finally, for this application, an investment budget of 3278880 TL and some security measures must be taken to prevent damage to the fence system designed with photovoltaic panels.

REFERENCES

Kumar, R., Rajoria, S.C., Sharma, A., Suhag, S. (2021). Design and simulation of standalone solar PV system using PVsyst Software: A case study. Materials Today, Proceedings, 46, 5322-5328.

Nwaigwe, N.K.P. Mutabilwa, P., Dintwa, E. (2019). An overview of solar power (PV systems) integration into electricity grids. Materials Science for Energy Technologies, 2, 629-633.

Sarraf, K.A., Agarwal, S., Sharma, K.D. (2016). Performance of 1 MW photovoltaic system in rajasthan: A case study, IEEE 7th Power India International Conference, 25-27.

Vaka, S.A., Talukdar, P. (2020). Novel inverse heat transfer technique for estimation of properties and location-specific process parameters of roof-mounted solar PV plants. Thermal Science and Engineering Progress, 19, 100657.

Kaleshwarwar, A., Bahadure, S. (2023). Validating the credibility of solar simulation tools using a real-world case study. Energy & Buildings, 301, 113697.

Oyshei, R.K., Hasan, S.M.K, Sadat, N., Hoque, A.M. (2024). Comparative analysis of solar module configuration and tracking systems for enhanced energy generation in South Sakucia Union, Bhola, Bangladesh: A software based analysis. Heliyon 10, e33884.

Sow, A., Mehrtash, M., Roussea, R.D. Didier Haillot, D. (2019). Economic analysis of residential solar photovoltaic electricity production in Canada. Sustainable Energy Technologies and Assessments.33, 83-94.

Ayadi, O., Al-Assad, R., Al Asfar, J. (2018). Techno-economic assessment of a grid connected photovoltaic system for the University of Jordan. Sustainable Cities and Society, 39, 93-98.

Hamad, J., Ahmad, M., Zeeshan, M. (2024). Solar energy resource mapping, site suitability and techno-economic feasibility analysis for utility scale photovoltaic power plants in Afghanistan.

https://next2sun.com/en/solar-fence/

LIFE CYCLE ASSESSMENT (LCA) OF BIO-ELECTROCHEMICAL SYSTEMS: AN EVALUATION FOR SUSTAINABILITY

Furkan Baş[1], Sevgi Demirel[2]
[1]Niğde Ömer Halisdemir University Faculty of Agricultural Sciences and Technologies Biosystems Engineering
[2]Niğde Ömer Halisdemir University Engineering Faculty Environmental Engineering

Corresponding author e-mail:furkanbas@ohu.edu.tr

ABSTRACT

Bio-electrochemical systems (BES) are eco-friendly systems that are being used for various of purposes such as water treatment, resource recovery, biogas and biohydrogen production from various of biomass resources. In the bio-electrochemical process (BEP), biomass is decomposed and break into smaller components by both biological and electrochemical reactions. The biological activities of the bacteria and electrochemical reactions involved in this process can cause some environmental effects. Moreover, waste materials resulting from the BEP have the potential to pose a hazard to the environment and public health. Therefore, a detailed life cycle assessment (LCA) covering the entire duration from raw material supply to the management of waste generated at the end of the process should be carried out and the environmental impacts of this process should be evaluated. LCA for a BES should be “Cradle to Grave” approach and include transportation of waste material, pre-treatment of waste, inflow of waste into BEP, reactions occurring in BEP, outputs (product and waste), processing-making usable-transportation of the product, processing-transportation-disposal or recycling of waste. In LCA, system boundaries should be defined clearly and comprehensively and environmental impacts should be analysed based on various criteria such as energy consumption, fuel type and consumption, greenhouse gas emissions, and toxic substances released into water and soil, to help design and manage products, services and waste in a more sustainable way.

Keywords: Bio-electrochemical systems, energy, environmental impact, life cycle assessment, sustainability.

INTRODUCTION

Bio-electrochemical systems

Bio-electrochemical systems (BES) are the promising technologies that can be used for electricity generation, synthesis of chemicals, production of biogas, biohydrogen, and biofuels, wastewater treatment, and resource recovery. In a BES, both biologic and electrochemical reactions occur at the same time. In the biologic reactions, bacteria and microorganisms take role and digest the organic material and decompose it. In the electrochemical reactions, electrical energy is used to break the chemical compounds to the smaller and shorter compounds. During and after bio-electrochemical process (BEP), various of end-products and by-products are produced. Some of the main BES are microbial fuel cell (MFC), microbial desalination cell (MDC), bio-photovoltaic systems (BPV), photosynthetic microbial fuel cell (photoMFC), enzymatic fuel cell (EFC), microbial electrolysis cell (MEC). Moreover, BES are promising technologies due to their specific advantages such as sustainability, scalability, energy efficiency, and versatility (Ahmadi, Rezae et al. 2023).

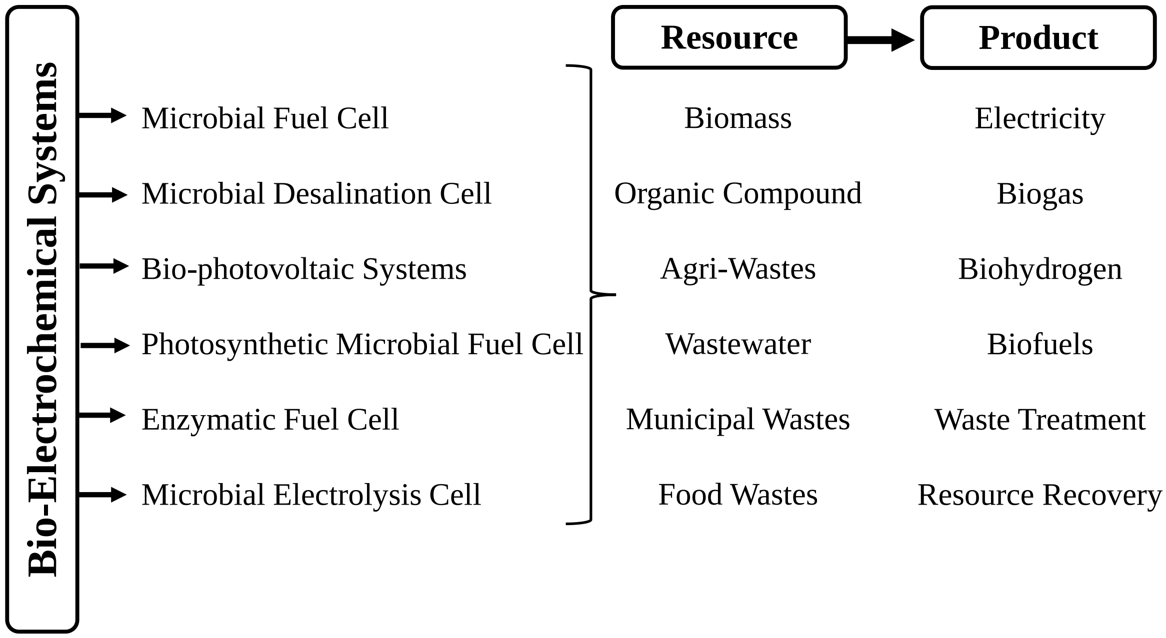


Figure 1. BES reactor, resources, and products.

BESs can be used for different purposes in many sectors. The operating conditions of BESs, the raw materials used, and the organisms involved in the process should be determined according to the intended use of the BES and optimized for the intended output. Table 1 shows the recent studies in which different BES types are used for different purposes.

Table 1. Recent studies on sustainable BES.

Organism	Substrate	Anode	Cathode	Reactor	Output	Ref.
Acorns tatarinowii	Silica sand + Water	Graphite	Pt-graphite	MFC	21 mW/m2	(Liu, Ji et al. 2018)
Mixed consortia	Petroleum refinery wastewater	Graphite	Pt-carbon	MEC	789 mW/m$_2$	(Mohanakrishna, Al-Raoush et al. 2019)
Sludge	Corn stover + Torrefied biomass	Carbon	Carbon	MEC	Bio-H_2 (123.72 mL/g TS)	(Li, Fan et al. 2022)
Anaerobic sludge	Sludge	Pt-Ir-Ti	Graphite	BES-AD	CH_4 (4.3 CH_4/ g COD)	(Jadhav, Yu et al. 2024)
Proteobacteria	Domestic wastewater	Carbon	Carbon	MDC	COD Removal (%55)	(Varjani 2022)

When Table 1 is examined, it can be seen that different organisms, substrates, anode and cathode materials, reactor setups and outputs are obtained. Organisms are actively involved in the biological reactions of BEP and the digestion kinetics of each organism are different from each other. In this context, it is important to create a synergy between the organism and the substrate to be used. If the substrate cannot be digested by the organism, it will disrupt the biological reactions of BEP.

The selection of materials used in electrodes is also a critical parameter for BESs. It is very important that ionic conductivity is high and ohmic resistance is low in the redox reactions that will occur between the electrodes and the substrate. In this context, when selecting the substrate, the electrical conductivity values of the waste should be taken into account, and in addition, the electrode material should be selected from materials with low internal resistance. In addition, electrodes should be selected from biocompatible, low-cost, corrosion-resistant, stable and safe materials. Therefore, the electricity production, biogas, biohydrogen, waste treatment and chemical treatments can be done with the help of BES. The fact that the raw materials used in these processes consist of organic waste and the system is environmentally friendly allows these outputs to be realized in a sustainable way.

However, along with advantages, BES's also has some disadvantages. These systems require technological equipment, knowledge of both biology and electrochemistry, system instabilities depending on the microbial activity, can be easily affected by environmental factors (temperature, pressure, humidity, light), impurities depending on biomass type, and environmental impacts. To prevent these disadvantages, biomass type should be selected carefully, operation conditions should be set meticulously, end and by-products should be observed, and waste management procedures must be implemented caution (Ramanaiah, Chandrasekhar et al. 2023).

LIFE CYCLE ASSESSMENT APPROACH in BIO-ELECTROCHEMICAL SYSTEMS

Life cycle assessment (LCA) is a systematic and holistic method used to assess all life stages of a product, from raw material (cradle) to production, distribution, use and disposal or recycling (grave), and the environmental impacts of these stages. It involves compiling an inventory of energy-material inputs and emissions, pollution and toxic substance releases of the product or service to be assessed, assessing the potential impacts of these inputs and releases on the environment, and interpreting the results to help make more informed decisions aimed at reducing negative environmental impacts. LCA provides a comprehensive analysis of environmental aspects and potential impacts throughout a product's life cycle, enabling a more sustainable approach to product design, development and management (Arvanitoyannis 2008).

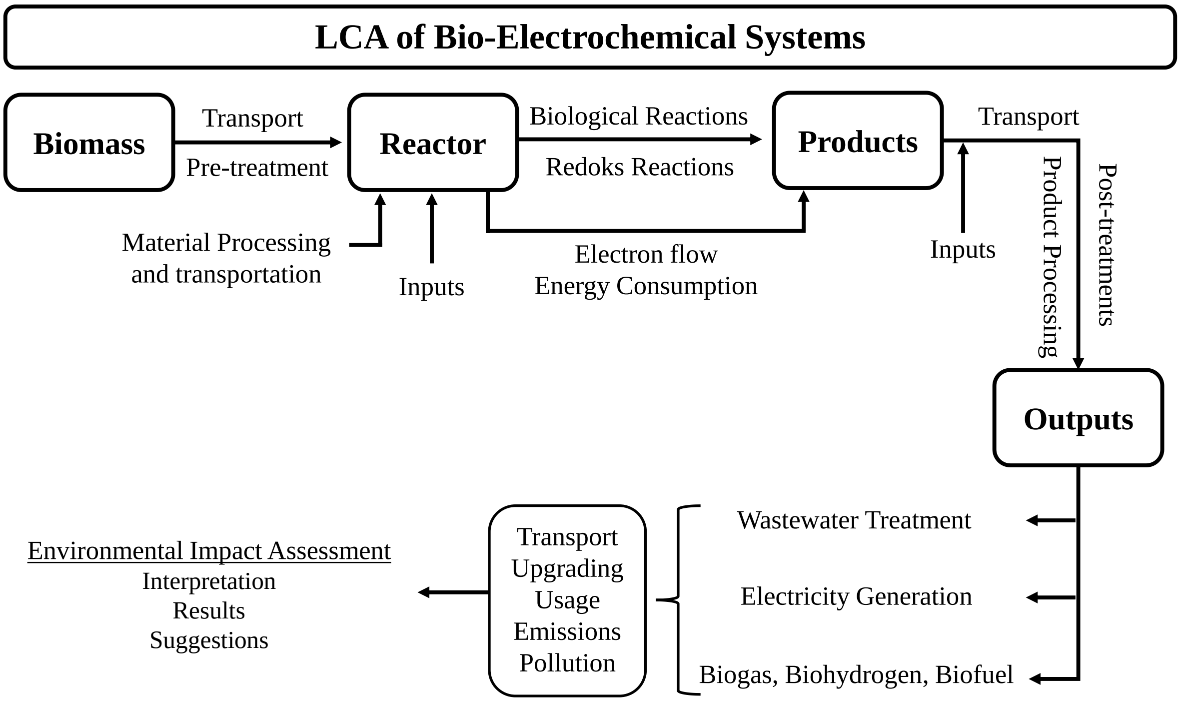


Figure 2. LCA of a BES.

As mentioned earlier, BES are innovative technologies that are used in various applications such as biofuel production, electricity generation, wastewater treatment and synthesis of valuable chemicals through the combined utilisation of metabolic activities of microorganisms and electrical energy. However, BESs also have serious environmental impacts and the sustainability of these systems is of great importance for the future utilisation of these technologies. To ensure the environmental and economic sustainability of these systems, LCA is used as a critical assessment tool. LCA can be used to systematically and comprehensively assess the environmental impacts of BES covering all stages from extraction of raw materials, transport, pre-treatment, inclusion in the production process, final and by-product production to final waste management. LCA can provide a sensitive, realistic and consistent assessment of the environmental impacts of BES in various environmental impact categories such as energy consumption, greenhouse gas emissions, water use, toxic substance releases. The application of LCA helps to identify areas for improvement in the design and operation of the BES, while at the same time enabling strategic decisions to be taken to achieve sustainability goals. Therefore, the relationship between BES and LCA is vital to minimise the environmental impacts of these technologies and ensure long-term sustainability. By providing a scientific and objective basis for optimising the environmental performance of BES, LCA contributes to the development of more sustainable and efficient bio-electrochemical applications (Savla, Pandit et al. 2021).

Stages of Life Cycle Assessment Studies in BES

1. Defining Goal and Scope

The aim of LCA is to realistically assess the environmental impacts of BESs. The study covers the life cycle of BES starting from raw material extraction to production, utilisation and waste management stages.

2. Inventory Analysis

Inventory analysis is carried out to detail the inputs used and outputs produced at each stage of the RPS. At this stage, various sources such as official data, literature reviews, producer data and laboratory measurements are utilised (Zhang, Yuan et al. 2019).

2.1. Inputs

- Raw materials: Anode and cathode materials (carbon-based, metal, ceramic materials), membrane materials (Nafion), cable and connection materials, nutrient media and microorganisms.
- Energy: All kinds of energy consumed during raw material pre-treatment and BES installation and BEP.
- Water: The amount of water required for the operation of the BES.
- Chemicals: Chemicals (phosphate buffer, nutrient solutions) used for raw material preparation, operation of the BES.

2.2. Outputs

- Electricity Generation: The amount of electricity generated during the operation of the BES.
- Biogas, biohydrogen production.
- BOD, COD, and heavy metal removal.
- Emissions: Greenhouse gases (CO, CO_2, CH_4, H_2S, NOx) released to the atmosphere during operation of the BES.
- Wastes: Waste and non-recyclable materials generated before, during and after the process.

3. Impact Assessment

In this stage of LCA, the environmental impacts of BES are analysed in various environmental impact categories using the data obtained from the inventory analysis. The main impact parameters analysed are as follows:

- Global Warming Potential (GWP): Impact of GHG emissions of BEP on climate change.
- Energy Consumption: Total amount of energy used during the installation and operation of the raw material and BES.
- Water Use: The amount of water consumed by BES throughout its entire life cycle.
- Toxicity: Potential impacts on the environment and living health of toxic substances released before, during and after operation of the BES, taking into account raw material pre-treatment.

4. Interpretation

By analysing the impact assessment results, inferences can be made on the environmental performance of BES. Furthermore, recommendations and suggestions are made to minimise the environmental impacts of BES. Recommendations include the selection of more environmentally friendly materials, measures to improve energy efficiency and waste management strategies (Chin, Phuang et al. 2022).

CONCLUSION

Life cycle assessment (LCA) of bio-electrochemical systems (BES) focuses on comprehensively assessing the environmental impacts of these sustainable and environmentally friendly green energy technologies. Similar studies and evaluations on this topic reveal that BESs can generate significant environmental impacts such as greenhouse gas emissions, high energy consumption, intensive water use and toxic substance release. To minimise such environmental impacts, environmentally friendly materials should be selected, strategies to increase energy efficiency should be identified and implemented, water-soil-waste management and energy recovery-recycling practices should be increased, and these measures should be taken, and toxic substances should be monitored. These recommendations provide concrete steps to improve the sustainability and efficiency of BESs by optimising their environmental performance.

REFERENCES

Ahmadi, S., et al. (2023). "A review on bioelectrochemical systems for emerging pollutants remediation: A computational approaches." Journal of Environmental Chemical Engineering **11**(3): 110021.

Arvanitoyannis, I. S. (2008). "ISO 14040: life cycle assessment (LCA)–principles and guidelines." Waste management for the food industries: 97-132.

Chin, M. Y., et al. (2022). "Life cycle assessment of bioelectrochemical and integrated microbial fuel cell systems for sustainable wastewater treatment and resource recovery." Journal of environmental management **320**: 115778.

Jadhav, D. A., et al. (2024). "Paradigm shift in NEW (Nutrient-Energy-Water) sustainable wastewater treatment system through synergy of bioelectrochemical system and anaerobic digestion." Bioresource Technology: 130404.

Li, Y., et al. (2022). "Pretreatment of corn stover by torrefaction for improving reducing sugar and biohydrogen production." Bioresource Technology **351**: 126905.

Liu, B., et al. (2018). "Anodic potentials, electricity generation and bacterial community as affected by plant roots in sediment microbial fuel cell: Effects of anode locations." Chemosphere **209**: 739-747.

Mohanakrishna, G., et al. (2019). "Removal of petroleum hydrocarbons and sulfates from produced water using different bioelectrochemical reactor configurations." Science of the Total Environment **665**: 820-827.

Ramanaiah, S., et al. (2023). "Bioelectrochemical systems (BESs) for agro-food waste and wastewater treatment, and sustainable bioenergy-A review." Environmental Pollution **325**: 121432.

Savla, N., et al. (2021). "Techno-economical evaluation and life cycle assessment of microbial electrochemical systems: A review." Current Research in Green and Sustainable Chemistry **4**: 100111.

Varjani, S. (2022). "Prospective review on bioelectrochemical systems for wastewater treatment: achievements, hindrances and role in sustainable environment." Science of the Total Environment **841**: 156691.

Zhang, J., et al. (2019). "Life cycle environmental impact comparison of bioelectrochemical systems for wastewater treatment." Procedia Cirp **80**: 382-388.

THE PATH OF ADVANCEMENT FOR FEMALE PHYSICIANS: FIGHTING AGAINST GENDER INEQUALITY

Gülden Aynacı[1]

[1]*Department of Health Science, Trakya Unıversity, Edirne, Turkey*

guldenaynaci@hotmail.com

ABSTRACT

Gender inequality is one of the most important problems faced by female physicians in the healthcare sector. Female physicians face various obstacles in their medical education and professional careers, and this can also affect the quality of healthcare services. In this article, we will discuss the current status of gender inequality for female physicians and the approaches developed to reduce this inequality. Female physicians may generally face more discrimination than their male colleagues in medical education. Female students are often subjected to prejudiced evaluations such as "weak" or "insufficient" in medical schools, which can negatively affect their self-confidence and academic success. In addition, female medical students and young physicians receive less mentoring support than their male colleagues. Female physicians have difficulty balancing work and family life. Childcare, housework and other family responsibilities in particular can directly affect female physicians' job performance and career advancement. In order to eliminate gender inequality, awareness should be raised on gender equality issues in medical education. Educational institutions should organize seminars and workshops for students on gender equality and combating discrimination. Supportive measures such as child care services and flexible working hours should be taken for female physicians to balance their work and family life. Such arrangements can enable female physicians to be more successful in their careers and have a more satisfying experience in their work life. Gender equality will improve the quality of not only women physicians but the entire healthcare system and create a more equitable working environment. The healthcare sector should take important steps to achieve gender equality and be a part of this process.

Keywords: Education and Awareness, Gender Awareness, Women Physicians, Gender Inequality, Medical Education and Gender, Career Advancement and Gender

INTRODUCTION

Gender inequality at work is an old and complex issue needing multidimensional analysis. Healthcare fields present similar problems of variousness in terms of rates throughout each occupational scale. The reasons may be related to some peculiar items that uniformly characterize the world of care but have been largely underinvestigated. There is an important body of evidence underlining how the intersection between age, gender, identity, and – if one has kids – motherly status may have significant implications for professional experiences and opportunities of physicians. Both societal norms and institutional prerogatives concurrently contribute to defining what should be a fair professional environment among the healthcare workforce. Qualitative analysis reveals distinct societal dynamics that act in the informants' narratives, producing two levels at which a discussion of gender inequality arises. The career advancement of female physicians often involves a more challenging process than that of male physicians. Women have more difficulty than men in advancing to leadership positions, and their competence is often questioned. Women's social role and the difficulties in establishing a work-life balance can hinder their career advancement.

Female physicians generally receive lower salaries and are exposed to more difficult working conditions than their male colleagues. Wage inequality can negatively affect women's motivation and commitment to their profession. In addition, female physicians' job security and promotion opportunities are often more limited than men's. The ascending path of women towards equality in the workforce has been defined by the successive overturning of restrictive gender policies. The figure of the doctor is at the peak of competing dualities. While the profession has undergone a social upgrade from a mere trade to an elite and cerebral occupation, women can provide nurturing care and reassurance, coinciding with the medical profession's expectations of a counselor and confidante. Societal beliefs and gender roles became a turning point in women's advancement in medicine. Societal stigmas and gender roles suggest that medicine is better suited for males with their allegiance to one duty and their brains, whereas women are seen as homemakers, focusing solely on family life and their nurturing qualities rather than tackling the intense medical formations. Although women were outliers, winning the approval of these renowned teachers when others believed in the limitations of the female skull brought them self-respect. Simultaneously, these beliefs were passed down, compelling both students and faculty to conform in agreement. Presently, women and men are indistinguishably shaped inside the womb.

Social structure and the cultural environment intersect to create situations wherein the bridging of gender gaps becomes the contributors to uneven enrollment and career advancement in medicine. The synergy of such an environment can be hampering for career progression for women in medicine.

RESULTS

The experiences of women in medicine often significantly differ from those of their male colleagues. Female doctors must clear higher interpersonal and emotional hurdles in their professional pursuits. They are faced with bias from the beginning, receiving more negative feedback than men during the hiring process. Additionally, there exists a significant gender pay gap, reflected in both the types and amounts of compensation male and female doctors receive.

Women in medicine also experience microaggressions and outright sexism, which add up to create a hostile and uncomfortable work environment. These disparities aren't just detrimental to female doctors' job performance—they can also contribute to feelings of impostor syndrome and self-doubt. Especially when it comes to leadership positions in academic medicine and medical organizations, female doctors must sometimes wait significantly longer than their male counterparts to be given either the responsibility of their male peers or the professional recognition that makes pursuing further education worthwhile. These delays can contribute to what has been called the leadership labyrinth, making the risk-reward of the substantial research and financial investment necessary to advance in these areas difficult to justify. Combined with existing cultural norms, which stereotype and undervalue both physicians' nurturing qualities and women in the workplace, the evidence suggests that the dual discrimination facing physicians who are both female and weigh in favor of their personal lives leads to decreased representation at higher levels in medicine. In turn, the degree of self-doubt and stress placed on physicians, and in turn, the powerful impact on one's career, are often compounded by work-life conflict, in which individuals feel that their vocational expectations conflict with family or personal pursuits. In a more effective response, all of those barriers must be quantified and addressed.

The issue of work-life balance is one of the most challenging ones in the life of female physicians. However, being important for men as well, it is inevitably women who bear the brunt of the responsibility. There is no doubt that both the nature of the medical profession and the journey towards the position of a physician are very stressful and often lead to burnout. As the expectations of society are still higher on women, being a female physician frequently elevates stress as well. Although in Hungary, women generally invest less time than men in paid work, the study of job burnout in a physician's community has shown that the phenomenon is more common among female doctors. This fact is explained by multiple inequalities and barriers that women's professional advancement must break through worldwide. There are also indications showing that stress and sleep disorders are even more severe for female physicians. Furthermore, this problem affects medical students as well. This is the key moment when those who reject the heavy overtime load arising from the engagement of the profession on the part of the employer leave the profession or adopt a lower workload. Women are less likely to choose positions that prevent them from having a healthy work-life balance. An unrealistic anticipation of economic growth is behind the lack of structural response in the developed world; that is, the nature of the work of male physicians has not changed even in the face of these trends, and women are simply expected to already do double responsibility while working longer.

A professional organization can function as a support system and advocate for female physicians. They provide several advantages to women and men who are members, as well as the medical community at large. Among the resources professional organizations offer are targeted online databases listing speakers and honorees, and resources for women in health care. Many organizations offer lectures, panel discussions, awards, and programs designed to increase mentorship, visibility, and leadership of females in healthcare. In general, joining a professional organization can enhance visibility and afford networking and mentoring opportunities. Female physicians are encouraged to become members of grant-making agencies and to target joining and succeeding within local, state, and national professional organizations. These committees and work groups can help raise the visibility of women and their issues in medicine, improve role modeling for future leaders, disseminate role-appropriate career and professional development resources, and change organizational culture. The Gender Equity Task Force has worked for almost 30 years to increase the equitability of evaluative and climate-related measures of success. These changes affect women, but they also help males in their careers. The ideation and concept for the mission and goals for this paper resulted from work in leadership roles within multiple professional organizations over the past two decades. These guiding principles have improved the organizations and the careers of both men and women. A favorite task was reviewing and voting upon the candidates for professional society senior membership.

Gender equality trainings and awareness-raising activities should be carried out for all individuals working in the health sector. These trainings can help both women and men become informed about gender inequality issues and combat these issues more effectively. Women physicians play an important role in the healthcare sector, but they face various challenges due to gender inequality. Comprehensive approaches and solutions should be developed to eliminate inequalities in areas such as education, career advancement, wage inequality, and work-family balance.

The mentoring programs should be established for female medical students. In order to provide equal wages and working conditions for female physicians, gender equality policies should be developed in the health sector. Legal regulations and policies combating gender inequality should be implemented in workplaces. In addition, incentives should be provided to support female physicians to advance to leadership positions.

Undergraduate and postgraduate medical training are important gateways that regulate, to some extent, the pathway between personal and professional development in the face of gender inequality. In an academic setup, the presence of bias influences the course of events, facilitating mentorship in favor of males. This is what we see occurring within higher education among faculty members. The presence of role models alone, combined with the social psychological principle of self-appearance, affects professional goals for students. Major interventions need to be made in medical school; the strategies for this will be discussed in detail later.

CONCLUSION

Women still need to overcome many types of gender injustice on the medical path to equality with men up to this day. These issues of justice can be tackled by addressing the level of resistance women feel to inequality in the workplace. Organizations and society as a whole can assist many women willing to stay in the workforce by understanding the invisible and tangible barriers that exist and hinder advancement into the profession of medicine, as well as personal activities outside of medicine. Stakeholders at the individual, organizational, and public policy levels must continue to work together to create an environment where women can have equality of opportunity, fair exposure, and fair compensation, rather than being expected to mimic male standards of success. There is reason to be optimistic since many actionable solutions are emerging. Advocates in various regions of the world propose a gender equality approach, with actionable advocacy reforms in mentorship, architecture, and education. As ideas for these reforms emerge from our gender and career path analysis, we find offerings potential stakeholders can accept so women receive fair outcomes. These differences that prescribe and proscribe what individual females should or should not bring from home to their careers and the workplace serve as our critical refutation to arguments that choose to pigeonhole the issue of unequal paths as an insufficient desire on the part of female physicians. With the benefit of the current knowledge base from successful appeals or unexpected revelations, researchers and implementers alike can attempt to subject laws, local and regional policies, and other reforms to reform and replication, data collection, and more educational theories, replacing those that failed, advancing and ultimately winning the fight. People, interest groups, institutions, and systems all play a critical part in the future of gender issues in medicine. Every day holds opportunity for better resolution, so we challenge the people today to continue the fight for tomorrow's advantage.

REFERENCES

Cleghorn, E. (2022). Unwell women: misdiagnosis and myth in a man-made world.

De Waal, A. (2021). New pandemics, old politics: Two hundred years of war on disease and its alternatives.

Jones, N. H. (2021). Our democracy's founding ideals were false when they were written. Black Americans have fought to make them true. The Best American Magazine Writing 2020.

Joseph, M. M., Ahasic, A. M., Clark, J., & Templeton, K. (2021). State of women in medicine: history, challenges, and the benefits of a diverse workforce. Pediatrics.

Korolczuk, E. (2020). Counteracting challenges to gender equality in the era of anti-gender campaigns: Competing gender knowledges and affective solidarity. Social Politics: International Studies in Gender, State & Society, 27(4), 694-717.

Saich, T. (2021). From rebel to ruler: One hundred years of the Chinese communist party.

Schuller, K. (2021). The trouble with white women: A counterhistory of feminism.

Sims, E. K., Carr, A. L., Oram, R. A., DiMeglio, L. A., & Evans-Molina, C. (2021). 100 years of insulin: celebrating the past, present and future of diabetes therapy. Nature medicine, 27(7), 1154-1164.

Smith, S. G. & Sinkford, J. C. (2022). Gender equality in the 21st century: Overcoming barriers to women's leadership in global health. Journal of Dental Education.

URINARY INCONTINENCE ISSUES IN WOMEN AFTER COVID-19

Gülden Aynacı[1]

[1]*Department of Health Science, Trakya Unıversity, Edirne, Turkey*

guldenaynaci@hotmail.com

ABSTRACT

The Covid-19 pandemic has had extensive effects on health and social life, and women's health has also been affected by these effects. The increase in urinary incontinence problems in women after the pandemic requires investigation of the causes and management strategies of this situation. This study examines in detail the causes of the increase in urinary incontinence problems in women after Covid-19, potential risk factors and treatment approaches. The Covid-19 pandemic has affected the health status of millions of people worldwide, as well as causing significant changes in women's health problems. Urinary incontinence is a common health problem among women and has become more pronounced with the impact of the pandemic process. This article will discuss the causes of the increase in urinary incontinence problems after Covid-19 and management strategies.

Keywords: Covid-19, Lifestyle changes, Women's health, Urinary incontinence, Post-Pandemic, Access to health care

INTRODUCTION

The effects of the pandemic on women's health are multidimensional: Physical Health: In addition to the direct effects of Covid-19, inactivity, weight gain and other health problems have also been experienced during the pandemic. This has affected women's physical health and paved the way for conditions such as urinary incontinence. Psychological Health: The pandemic has caused an increase in stress, anxiety and depression levels. Psychological health problems can affect bladder control and increase the risk of urinary incontinence. Access to Healthcare: Access to healthcare has become difficult during the pandemic, causing disruptions in the management of chronic health problems such as urinary incontinence.

Urinary incontinence is defined as the involuntary leakage of urine. There are three main types in women: Stress Type Urinary Incontinence: Occurs when the pressure on the bladder increases during physical activity, coughing or sneezing. Urgent Urinary Incontinence: Occurs as a result of sudden and strong contractions of the bladder, usually associated with a sudden and uncontrollable need to urinate. Mixed Urinary Incontinence: Shows both stress and urgent symptoms.

Reduction in Physical Activity: The obligation to stay at home and social isolation have caused a decrease in physical activity. Inactivity can lead to a weakening of the pelvic floor muscles and therefore an increase in the risk of urinary incontinence. Weight Gain: Weight gain was observed during the pandemic period due to changes in eating habits and physical activity. Obesity is an important factor that increases the risk of urinary incontinence. The pandemic process has caused an increase in stress and anxiety levels. This situation can have negative effects on bladder control and trigger urinary incontinence. Access to healthcare has become difficult due to the pandemic, which has led to a delay in the timely diagnosis and treatment of health problems such as urinary incontinence. Hormonal changes after menopause and childbirth can increase the risk of urinary incontinence. During the pandemic, women may have difficulty accessing the necessary healthcare services during these periods.

Managing urinary incontinence problems usually requires a multidisciplinary approach. Kegel exercises strengthen the pelvic floor muscles and can reduce urinary incontinence problems. Biofeedback: This method can help understand and control how the muscles work. Psychological Support: Stress Management: Stress and anxiety management can reduce urinary incontinence problems. Therapies and counseling services can provide support in this regard.

Cognitive Behavioral Therapy: This therapy method can help develop stress coping skills. Medications that relax the bladder muscles and control urinary incontinence may be used. In severe cases, surgical options may be considered. These methods may include bladder or urethral support procedures. Exercise and Weight Management: Regular exercise and weight control can reduce the risk of urinary incontinence. Dietary Adjustments: Regulating water intake and avoiding foods that can irritate the bladder, such as caffeine, may be helpful.

RESULTS

Urinary incontinence (UI) is highly prevalent in women, affecting about one in three. The global prevalence rate of UI is approximately 200–400 million, with an estimated worldwide cost of $65–77 billion annually, as UI has significant economic, health, and social consequences. In virtually all UI management guidelines, pelvic floor muscle training is considered the first-line therapy, emerging as an effective noninvasive treatment option. The pathophysiology is often multifactorial (e.g., menopause, hormone alterations, pregnancy delivery, trauma, and urinary tract infections). Evidence shows that women are at higher risk for severe respiratory syncytial virus and influenza virus infections, especially if they are pregnant, due to the widespread use of sex steroids in this population. Epidemiological data on the impact and pathophysiology of the novel coronavirus disease have been published, but there are unknown long-term complications. It is known that a woman’s immune response is higher than a man’s, which could have a harmful effect on patients with excessive immune response to SARS-CoV-2 infection. Promising studies are showing that female sex hormones are very important in preventing and supporting anti-sepsis responses due to lower serum testosterone in men, and the central nervous system immune regulatory role of estrogen and progesterone, which downregulates cytokine production. Due to its pathophysiology, complications, and high prevalence, we aimed to evaluate whether women who have COVID-19 have or will develop UI by evaluating the EMG activity of the pelvic floor muscle in the suspicion or diagnosis phase of COVID-19.

The SARS-CoV-2 (COVID-19) coronavirus pandemic continues to impact the lives of individuals at a global level. At this stage, literature on the impact of COVID-19 on lower urinary tract symptoms is scarce. However, the risk of urinary incontinence, predominantly in the older population following a COVID-19 infection, potentially has immediate and far-reaching welfare and economic consequences. A significant potential physical and psychological comorbidity due to COVID-19 infection is through disruption of the pelvic floor muscle system, leading to urinary incontinence. An increase in pneumonia, a potential outcome of a COVID-19 infection, may also predispose the development of urinary incontinence.

Several medical observations provide some explanations of how SARS-CoV-2 negatively affects pelvic floor muscle function. A relationship between COVID-19 infection and pelvic floor impairment mediated by the cytokine storm, with the lower urinary tract symptoms including low urinary stream, frequent urination, weak stream, painful urination, and incontinence is strongly suspected. It is reasonable that COVID-19 pneumonia may trigger a stimulus urge to diaphragmatic contraction via a stretch reflex elicited by alveolar distension and reduced lung compliance. This urge to diaphragmatic contraction decreases intra-abdominal pressure transmission to the pelvic floor muscles through a delay in pelvic floor muscle activation, which is a predisposing factor for urinary incontinence development. In addition, recent reports of prostate infection and the possible transmission of the coronavirus through seminal fluid and/or urine indicate some warnings in developing and implementing strategies to promote physical health and well-being for adult women who have been infected by SARS-CoV-2. There is a lack of evidence assessing combined conservative management and therapies for women developing urinary incontinence post COVID-19 infection. At present, there is no efficacy in using both together. However, the use of conservative management prior to prescription or any therapy in these women will improve the signaling through behavior, i.e., sensation to inhibit and initiate bladder emptying, restore a stimulated regular micturition habit, and reduce the possible relapse effect associated with antimuscarinic therapy. In the future, studies should examine COVID-19 women completely adhering to conservative therapy with the addition of selective drug therapies. In this clinical scenario, there are significant uncertainties about other selective medical therapy effects, ignoring valsalva non-classic voiding leakage or valsalva associated with storage orgasmic leakage, for example, which could also be considered. The most concerning uncertainty is the need to provide a longer-term follow-up in COVID-19 affected incontinent women beyond the bladder health program, due to the possibility of other illnesses due to virally established vascular and nervous damage.

It is important to remember that women experience different presentations of urinary incontinence due to their unique pelvic structures. Consequently, there are several surgical procedures to address their individual requirements. When counseling women post-COVID-19 infection and considering surgical treatments for urge urinary incontinence, it is important to evaluate patient suitability. Burch colposuspension has a good long-term cure benefit, but evidence from before the mesh era is increasing its acceptable use. Women aged 40 and over with no immediate desire for further children or who have completed their families are most suitable. Providing careful pre-operative counseling and informing patients about available alternative procedures, including retropubic and transobturator mid-urethral slings, which also have good short and medium-term outcome data, aids decision-making. It is important to counsel women about the possible post-operative de novo urge incontinence, as well as the need for concomitant mid-urethral slings during pelvic organ prolapse repair. Mid-urethral slings should be performed for suboptimal success because the alternate urodynamic approach is not likely to substantially influence the final surgical approach. When discussing surgical treatments for stress urinary incontinence following a COVID-19 infection, it is important to assess patient suitability for the procedures to ensure good patient satisfaction. Age and comorbidity are significant factors. However, counseling patients carefully before undergoing surgery and informing them of other surgical alternatives aids in the decision-making process. It is important to counsel women about the possible occurrence of de novo urge incontinence post-sling and to discontinue the mid-urethral sling at the time of the pelvic organ prolapse repair. Do VRAM slings demonstrate greater efficacy than TVT? The subject is then covered by a scientific discourse. These surgeries stress the significance of associating in discussion within pelvic floor multidisciplinary teams and making sure that the judicious approach offers as much effective prevention as it provides treatments to optimize outcomes simultaneously. However, a further randomized controlled trial is warranted.

The problem of urinary incontinence must always be addressed in a comprehensive understanding of the woman’s life and in the context in which she is inserted, whether they are personal, family, residential, educational, and/or work relationships, as well as emotional and love relationships and her free social life, absorption, and sexual transmission. The social restrictions associated with the infection may affect each affected woman in various dimensions, whether it is the impossibility of performing health care or the displacement to seek treatment, individual or group leisure activities, educational dynamics, the ability to work, engaging in studies, shopping, and household tasks, or in religious behaviors. These limitations may also factor in gender inequalities based on the fact that the role of primary caregiver for dependents is more often played by women. Such socially ingrained influences affect the Western femininity model and female self-representation, which are influenced by characteristics and behavioral patterns of women, with values and interests absorbed and incorporated from their perspective and mainly related to care for the body with outward and genital identity provided by the muscles of the perineal floor, body shape, and flawless skin, which are pre-established based on multiple sources and conflicts, producing paradoxical distress and pleasures. The intimate values that female patients develop, where the self is valued through continuous disclosure of herself, will have a direct impact on her sexual health, and the benefits and suffering provided and caused by the woman as a woman with the construction of her identity. The construction of her own meaning, without affecting the meaning it carries. This illness is part of a medicalization that must be addressed, not investigated in the abstract, because it is immersed in political, economic, social, and gender issues in the public and private dimensions.

Quality of life considerations are paramount in the management of urinary incontinence. There is increasing recognition that urinary incontinence may be bothersome and have a negative effect on a woman’s quality of life. It has been shown to adversely affect emotional well-being, physical, and social functioning in a highly symptomatic population of women, with women presenting with urinary incontinence and an overactive bladder having a similar quality of life impairment as other chronic conditions. Area-based cohorts have demonstrated that increasing the number of urinary incontinence episodes is directly associated with a poorer quality of life, and individuals with a more bothersome incontinence score a lower quality of life. Nocturnal urinary incontinence has been reported to have the greatest adverse effect on quality of life, with individuals experiencing higher levels of emotional distress and an overall impact on all quality of life domains.

Information on long-term sequels as well as data from over 50,000 recently recovered confirmed COVID-19 patients has almost just been analyzed. The importance of early rehabilitation in local communities becomes even more urgent in the face of this massive, growing, and permanent chronic illness. Of these, there were also more patients with proven previous urinary incontinence and new urinary incontinence following a COVID-19 infection. Furthermore, the ratios of new instances of urinary incontinence are disproportionately likely to occur in those without previous urinary incontinence. During primary and secondary prevention of worsening urinary incontinence, the role of physical activity and rehabilitation-based intervention in the local community should not be underestimated in the early stage of rehabilitation, especially in women.

Understanding the influence of COVID-19 could help in better rehabilitating patients following COVID-19 where urinary incontinence is concerned. During both primary and secondary prevention, it is important that high-quality guidance is provided regarding physical load and occupational stimulation that can be given safely. In this large cohort of women surviving a COVID-19 infection, the risk ratios were significant. What is needed immediately are further studies. Information on long-term sequels as well as long-term PTSS and PTSD studies is thus warranted. A previous history of urinary incontinence would significantly increase the risk of new COVID-19 urinary incontinence, and some socio-economic groups of women are particularly vulnerable to this.

CONCLUSION

Urinary incontinence (UI) is independently associated with increased risks of other major bladder and bowel symptoms in women following a recent COVID-19 infection and may be a vital symptomatic marker. Unrecognised sequelae including UI may contribute to increased healthcare usage and lead to irreversible morbidities. Importantly, there are safe and effective treatments for incontinence that can be initiated without the need for hospital referral and further invasive testing. Further work is needed to evaluate the strength of the association between UI and COVID-19, and if the presence of UI following a recent infection acts as a prognostic factor for other bladder or bowel related symptoms or indeed long-haul COVID-19. Establishing UI as a potential predictor could therefore avoid potential over-referral for vitally important lung function clinics, including prolonged wait times. Future work could also investigate if other novel therapies could help improve UI following a COVID-19 infection. Medics evaluating patients following infection should be alerted to the potential for UI and be confident in the safety and efficacy of the range of successful management options, targeting symptom improvement for both conditions.

REFERENCES

Cardoso, J. R., Steffanello, L. N., Ferreira, L. F., Leites, G. T., Da Rosa, P. V., & Rosa, L. H. T. D. (2024). Prevalence of urinary incontinence and associated factors in patients with post-COVID-19 syndrome after hospital discharge. Revista Gaúcha de Enfermagem, 45, e20230283.

de Castro Sousa, F., Estevam, L. F., Silva, E. P., Mayers, N. A. J., & Cupertino, M. C. (2023). Possible association of urinary incontinence with post-COVID-19: a report of three cases. The Journal of Infection in Developing Countries, 17(11), 1544-1548.

Farooq, M., Faisal, S., Rashid, H. H., Sharif, K., Habib, H., Khalid, M., & Hassan, Z. (2023). Frequency of Urinary Incontinence Among Post COVID Males. Journal of Health and Rehabilitation Research, 3(2), 174-180.

Ferrari, A., Corazza, I., Mannella, P., Simoncini, T., & Bonciani, M. (2023). Influence of the COVID-19 pandemic on self-reported urinary incontinence during pregnancy and postpartum: A prospective study. International Journal of Gynecology & Obstetrics, 160(1), 187-194.

Grimes, C. L., Balk, E. M., Crisp, C. C., Antosh, D. D., Murphy, M., Halder, G. E., ... & Meriwether, K. V. (2020). A guide for urogynecologic patient care utilizing telemedicine during the COVID-19 pandemic: review of existing evidence. International urogynecology journal, 31, 1063-1089.

Hillery, S. (2020). Taking care of continence patients during the COVID-19 pandemic. British Journal of Nursing.

Siracusa, C., & Gray, A. (2020). Pelvic floor considerations in COVID-19. The Journal of Women's & Pelvic Health Physical Therapy, 44(4), 144-151.

Yildirim, M., Minobes-Molina, E., Oliveira, V. R., Coll-Planas, L., Moreno-Martin, P., Rierola-Fochs, S., ... & Jerez-Roig, J. (2023). How did the COVID-19 pandemic affect urinary incontinence and its management in the nursing homes? A descriptive phenomenological study. Neurourology and Urodynamics, 42(2), 409-418.

RELATIONSHIP BETWEEN SEXUAL DYSFUNCTION (SD) AND RESTLESS LEGS SYNDROME (RLS) IN WOMEN: A CURRENT LITERATURE REVIEW

Gülden Aynacı[1]

[1]*Department of Health Science, Trakya Unıversity, Edirne, Turkey*

guldenaynaci@hotmail.com

ABSTRACT

Female sexual dysfunction (FSD) is a broad spectrum that includes various problems such as sexual arousal, orgasm and discomfort during sexual intercourse. Restless legs syndrome (RLS) is a neurological disorder characterized by discomfort and a need to move the legs, usually while lying down or at rest at night. The relationship between these two conditions may be of interest in terms of both physiological and psychological factors. RLS is characterized by discomfort and a feeling of restlessness in the legs. These symptoms usually occur in the evening or at night and are relieved by the need to move. RLS is generally more common in women and can significantly affect sleep quality. The prevalence of RLS varies between 5-10% in the general population, but this rate may be higher in women. RLS is thought to be caused by genetic, biochemical and environmental factors. SD includes problems such as sexual arousal, orgasm, and discomfort during sexual intercourse. Types of SD include sexual desire disorder, sexual arousal disorder, orgasmic disorder, and sexual pain disorder. SD is common among women and is seen in various age groups. The prevalence of SD may vary depending on the age of the women and their physical and psychological health status. There may be a psychological link between RLS and SD. Constant discomfort and insomnia may increase the risk of anxiety and depression. These psychological conditions may contribute to sexual dysfunction. The relationship between RLS and SD in women requires a multidisciplinary approach. Treatment strategies should be developed considering the effects of both physiological and psychological factors. Understanding the effects of RLS on sexual function is important in terms of coping with this condition and developing treatment strategies. The literature on this subject is still developing and more comprehensive studies are needed. In particular, longitudinal and control group studies may be useful to understand the mechanisms of the relationship between RLS and SD. Research on this subject is very important in terms of its effects on women's sexual health.

Keywords: Female Sexual Dysfunction (SDY), Restless Leg Syndrome (RLS), Sexual Dysfunction, Hormones and Sexual Function, Women's Health

INTRODUCTION

Restless legs syndrome (RLS) has lately been reviewed to have a relationship with different diseases, both in terms of organic and psychiatric diseases. In addition, in the etiopathogenesis of the mentioned diseases, organic and psychiatric causes like metabolic, hormonal, and ischemic causes play a role in the pathways. It has been noted that RLS is associated with sexual dysfunction. There is not enough data on RLS's etiopathological status. Therefore, the studies that would be conducted are very significant in terms of practical and social developments on RLS that could be performed by the findings, prevention, and treatment of the illness.

RLS is known to be associated with sleeplessness and constant restlessness at night. This condition can affect the general quality of life and lead to sexual dysfunction. Insomnia can cause symptoms such as lack of sexual desire and fatigue.

According to the current data, the relationship between RLS and sexual dysfunction is also known throughout the prevalence studies. The low quality of the investigations has been noteworthy when evaluating the relationship between RLS and sexual dysfunction. Additionally, the distinguishing symptoms or the difficulties of sexual dysfunction related to RLS could not be clarified in the studies when the comorbidities of RLS were mostly excluded or not. In a multitude of RLS cases, when the RLS symptoms before and during sexual activity were questioned, the sleep and feelings of restlessness were negatively affected. During sexual activity, the interactions of the proper physical and emotional symptoms and sexual dysfunction of RLS patients negatively impacted their desires, while the possibility of sexual dysfunction increased when RLS symptoms during sexual intercourse were added to the emotional factors. Women suffered from inability to sleep, instability, anxiety, sexual incompatibility, and depression. When RLS was included, a significant increase in the possibility of difficulty falling asleep or having agitated sleep was reported. The role of dopamine was evaluated in the sexual processes as well. A certain medication was reported to have a sexual side effect as an inhibitor agent on the dopaminergic transmission, and, as known, certain effects are well-established. The results of the therapeutic treatment with inhibitors of the dopaminergic transmission were variably reported. While some studies stated that a significant percentage of some dopamine agonists suppressed sexual desire and orgasmic response, others countered the claim that they had no effect on the scores of sexual function. As a result, it has been determined that RLS and dopaminergic treatment have a negative impact on sexual dysfunction in women with or without RLS.

RESULTS

Sexual life has an important impact on a couple’s life, playing a significant role in interpersonal relationships. Sexual dysfunction is an important source of distress, with relevant clinical implications. It often causes interpersonal relationship difficulties and contributes to a reduction in the quality of life. Furthermore, sexual relationships are an important goal for human beings, with a role in their general functioning and well-being. There are, however, limited data about restless legs syndrome and its relationship with female sexual dysfunction. This review aims to evaluate the current state of research on sexual dysfunction in women with restless legs syndrome. Restless legs syndrome is a very common sensorimotor disorder. It frequently leads to a reduction in the quality of life and has been found to have a significant impact on sleep, mood, exercise, and social activities. Female sexual dysfunction is characterized by significant difficulties experienced during one or more of the sexual responses. Its importance is due to the fact that it might be considered an expression of general health problems, such as endocrine, neural, vascular, or emotional disorders, and, therefore, it makes sense to subject these patients to specific investigations in order to identify any underlying causes.

The substantial evidence from neuroscience and psychophysiological research on the role of dopamine in preparing for and promoting sexual motivations supports the aberrant functions of the dopaminergic system in the occurrence of sexual dysfunctions and the high prevalence of restless legs syndrome in women. One suggestion has been to consider evaluating dopamine levels or urinary dopamine excretion in such women. Because obesity has been linked to other sexual dysfunctions, weight maintenance could also be considered as an option to prevent or improve sexual dysfunction due to restless legs syndrome. In the case of psychological mechanisms, attention and cognitive function, estrogen levels, and the expectations of sexual pleasure may contribute to female sexual function through physiological pathways. The co-occurrence of sexual dysfunctions and restless legs syndrome is believed to result from frequent daily stressors or aging, as suggested by the findings that one in three women with restless legs syndrome experience sexual dysfunction and depressive mood. Both sleep disturbances, due to periodic limb movement disorder, and low sex hormone levels resulting from reduced energy due to low sleep quality, could impact the sexual function of regularly stressed women by causing and aggravating anxiety and pain concomitantly. Disturbances in restorative sleep due to alterations in the release and metabolism of neurotransmitters in affected brain centers, similar to what occurs in sexual dysfunction, have also been suggested as potential mechanisms.

CONCLUSION

The significant impact of RLS on the well-being and health-related quality of life has been recognized widely. RLS primarily causes adverse effects on subjective sleep quality and daytime functioning, including activity impairment, work productivity loss, and negative effects on mood and sex life. The presence of RLS engenders higher functional disability, increased medical care utilization, and a reduced quality of life in women. Therefore, we can speculate that the presence of a concurrent physical and/or mental health problem, such as impacts on relationships, partners, and family, employment loss, and decreased productivity, could initiate difficulties in sexual function, even if the physiopathology of RLS is not directly involved. In this context, RLS can result in negative effects on sexual life, while less attention has been paid to the sexual dimension of RLS despite the significantly reduced health-related quality of life. RLS causes unpleasant sensations in the limbs, and the resulting discomfort is exacerbated at rest and relieved by active movement, for example, walking or stretching. In many cases, the symptoms occur in the evening and at night, causing patients difficulties in initiating and maintaining sleep, resulting in sleep disturbances with a substantial impact on the patients' behavior, relationships, and overall quality of life. Given the diurnal and nocturnal symptoms, RLS can affect couples' lives significantly, but information on sexual life has been mostly reported secondarily. The current review is not aimed at systematically analyzing the evidence of the negative effect of RLS on sexual life and couple relationships, but to speculate on the potential mechanisms of this sexual impairment.

Various studies have suggested that gabapentin and opioid antagonists may improve RLS symptoms, and the use of these medications for women with RLS who do not want to interfere with their sex lives could be considered. For example, gabapentin enacarbil sometimes causes headache, dizziness, and sedation, demonstrating a profile similar to that of the antidepressants proposed as a second-line treatment for SDY.

Personalized treatment strategies are also essential to improve symptoms, reinforcing the importance of communicating effectively with patients to ensure they feel comfortable discussing any sexual difficulties that may have developed over time. To our knowledge, useful recommendations for the treatment of symptoms associated with both disorders would also be helpful, and it may be feasible to meld two clinical guidelines on a multimodal treatment method. Patients with this comorbidity would prefer to receive treatment simultaneously rather than sequentially; that is, so they only have to see one physician for all their conditions rather than having to consult various specialists. This recommendation might be particularly applicable for some women diagnosed with both conditions.

REFERENCES

Agrawal, O., Panwar, V. K., Singh, G., & Mittal, A. (2022). Sleep, sleep disorders, and sexual dysfunctions. In Sleep and Neuropsychiatric Disorders (pp. 497-521). Singapore: Springer Nature Singapore.

Diaz, S., Abad, K., Patel, S. R., & Unruh, M. L. (2021). Emerging treatments for insomnia, sleep apnea, and restless leg syndrome among dialysis patients. Seminars in Nephrology.

Dilixiati, D., Kadier, K., Laihaiti, D., Lu, J. D., Rezhake, R., Azhati, B., & Rexiati, M. (2023). The relationship between sleep disorders, quality, and duration and sexual dysfunction: a systematic review and meta-analysis. The Journal of Sexual Medicine, 20(6), 766-780.

Manga Günaydin, F. & Tunç, A. (2024). Prevalence of restless genital syndrome and its impact on quality of life in women with restless legs syndrome. Neurological Research.

Salib, M., Memon, A. N., Gowda, A. S., Rallabhandi, B., Bidika, E., Fayyaz, H., & Cancarevic, I. (2020). Dialysis patients with restless leg syndrome: can we relieve their suffering?. Cureus, 12(8).

Zwerling, B., Keymeulen, S., & Krychman, M. L. (2021). Sleep and sex: a review of the interrelationship of sleep and sexuality disorders in the female population, through the lens of sleeping beauty syndrome. Sexual Medicine Reviews, 9(2), 221-229.

CURRENT LITERATURE REVIEW ON MENSTRUATION-RELATED MIGRAINE

Gülden Aynacı[1]

[1]*Health Sciences, Trakya Unıversity*

guldenaynaci@hotmail.com

ABSTRACT

Migraine is the second leading cause of disability globally, affecting women 2 to 3 times more than men. Migraine stands out as the single largest factor leading to the loss of years of healthy life, especially in women of childbearing age. Menstrual cycle-related migraine (MM) constitutes an important subgroup in this context, occurring in approximately 18% to 25% of women. MM is an important component of migraine that affects women's quality of life. This literature review will address current issues encountered in the diagnosis and management of MM and innovations in the literature. The ICHD-3 diagnostic criteria have some limitations in defining menstrual-related migraine. For example, the criteria do not appear to adequately address migraine frequency and timing. First, the criteria required by ICHD-3 require migraine to occur in two out of three consecutive menstrual cycles. However, this may lead to misdiagnosis in women with high frequency of migraine attacks, i.e., those with chronic migraine (CM) or high frequency episodic migraine (HFEM). This may affect the diagnostic criteria, as women with 8 or more migraine days per month must have a migraine attack within the perimenstrual window, simply by chance. Cases defined as rare pure MM refer to rare migraine attacks that occur only during the menstrual period. Migraine, and especially menstrual-related migraine, causes significant disability and loss of quality of life in women. The current ICHD-3 criteria present some difficulties and uncertainties in the diagnosis of this type of migraine. However, hormonal treatments and other medical approaches offer important steps in migraine management. Future research and updating of diagnostic criteria are critical for a better understanding of migraine and the development of effective treatment methods.

Keywords: menstrual-related migraine, women's health, migraine attacks, perimenstrual window, women's quality of life

INTRODUCTION

Of particular interest to researchers and clinicians in the field of women's health is menstruation-related migraine (MRM). This affliction, which presents with a predilection for menstruating women, has been the subject of increased attention in the literature over the past several decades. The necessity of its documentation has been varied, owing to an interest in symptom presentation, rate of occurrence, socioeconomic significance, and associated complications with contraceptive therapy. Accordingly, notable effort has been made to demonstrate, through rigorous scientific investigation, the importance of MRM's distinctions over and above migraine outside of the menstrual cycle. As will be extensively addressed below, current literature on this topic is primarily preoccupied with demographic characteristics, clinical presentation, and management strategies to evoke positive patient outcomes.

Recognition of menstruation-related migraine (MRM) is especially important as this type of headache is estimated to affect a significant 72.9% of females in the United States, with a somewhat decreased prevalence occupying the rest of the world (48.2%). Such niche diagnostic categories can hold significant implications for overall headache prevalence at approximately 11.7%. As such, our desire to document the experience of such a large population of potential patients is somewhat of a stretch. Nonetheless, MRM represents the processes of several cycles and complements our understanding of headache conditions—particularly as MRM patients can expect to spend 30% of their lifetime experiencing migrainous symptoms.

The current literature points to the observation that, in addition to menstruation-induced attacks, hormonal effects appear to be associated with the onset of migraines in initial cases as well as the course of chronic headache.

RESULTS

Menstruation-related migraine (MRM) is classified as migraine without aura but is associated with at least one menstruation-related episode of migraine without aura in at least two out of three menstrual cycles. In contrast to menstrual migraine, which refers to migraine without aura fulfilling the above symptom criteria occurring from two days before to three days after the onset of menstruation (day 0), MRM can occur at any point during this period. MRM is a universal phenomenon observed across racial and ethnic boundaries. When and how often it occurs shows considerable variability across and within populations, translating into individual risk for those affected. Most literature has focused exclusively on the female sex as subjects, specifically ascribing it to ovarian menstruation, reproduction, and contraceptive practices.

These cases may be difficult to accurately identify and manage because they do not have migraine attacks frequently enough to be diagnosed according to current criteria. In addition, there is a lack of clarity regarding the timing of migraine attacks. The term "occurring" in current criteria does not clarify whether they begin or end on days 1 ± 2 of the menstrual cycle. This uncertainty is a significant barrier to accurate diagnosis and treatment. In recent years, some innovations have emerged in the treatment and management of MM. Hormonal therapies play an important role, especially in the management of menstrual cycle-related migraine. Methods such as oral contraceptives and hormone replacement therapies can reduce migraine attacks in some women. In addition, various drug therapies, antidepressants and antiepileptic drugs are used to reduce the association of migraine with the perimenstrual period. These approaches may be especially effective in managing menstrual cycle-related migraine. Current research is conducting various studies to improve current diagnostic criteria and to make more accurate diagnoses. For example, large population-based studies and cohort studies are important to improve the definition and management of menstrual-related migraine. In addition, research on the role of biomarkers and genetic factors supports a better understanding of migraine and the development of personalized treatment approaches.

Prevalence in adult females Estimates from the general adult female population place the prevalence of MRM at 7–19%. Adolescent MRM attacks per patient per year occurring in individuals under 21 years of age were associated with a relative risk of 2.2 compared to adults. Women with MRM were shown to have a 1.33-fold increased risk of developing migraine in the future compared to women without MRM, after adjusting for age, hormonal contraceptive use, smoking, and migrainous family history. Women who frequently experience perimenstrual or menstrual-like headaches were overrepresented in patients presenting to a headache center. Compared to family doctor consultations, which demonstrated lower rates of MRM consultation, the proportion of women with a migraine onset around puberty was higher among headache center attendees. These individuals will often have a heavier individual and family burden of migraine. A woman's healthcare provider can use this data to provide patient education and explore individual maternal history and present comorbid health conditions.

Menstruation and hormonal headache are a leading cause of disability worldwide, and at a population level, more disabled days result from menstrual or hormonal headache than from any other disorder. Despite the current pandemic status, menstruation-related migraine is a major global public health issue. In contrast, non-migraine headache, including menstrual tension-type headache, did not have a negative impact on health.

There is extreme variation in the reported prevalence rates of menstruation-related migraine; this variability may be the result of regional differences, unstandardized populations, or the use of unreliable data. A meta-analysis of data from multiple studies reported pooled median rates of menstruation-related migraine of 17.0% and 5.5%. The upper range of the reported prevalence rates clearly indicates the extent to which this problem has been ignored in the wider public arena over recent years.

Potential risk factors for the development of menstruation-related migraine that have been investigated include both demographic factors such as sex, older age of onset of menstrual bleeding, greater number of pads required during menses, or a history of severe acne, alcohol, or chocolate craving. That cyclic hormonal changes can precipitate migraine in susceptible individuals has been appreciated for decades and is highlighted in the current classification for headache disorders. Estrogen has been shown to be essential for the development of menstrual migraine attacks, and therefore, many women who experience these episodes are candidates for preventive therapy. The role of lifestyle factors such as diet has not been fully explored in relation to hormonal headache or menstruation-related migraine. Stress of moderate to moderately severe intensity in women who have a menstrual attack is often mentioned negatively. Furthermore, women with menstruation-related migraine have a higher incidence of common medical comorbidities than those who do not. Long-term increased risk of depression and generalized anxiety disorder, stroke, and myocardial infarction is modestly but significantly increased in women with a history of migraine with or without aura. Women with a history of migraine at ages 40-74 have subclinical vascular brain injury as evidenced by imaging scans.

DISCUSSION

Menstruation-related migraines can significantly impair a woman's or a person's quality of life and productivity. The migraines are often described as severe and debilitating, interfering with daily activities and performance at work or school. It has been recognized that people suffering from chronic migraines report decreased quality of life and experience anxiety, depression, hopelessness, and several other comorbid medical conditions. Menstrual migraines and the often unpredictable early attack can cause significant emotional distress, difficulty concentrating, anger, and depression in affected persons, all of which can interfere with daily activities, work, productivity, and leisure. A close association between the severity or frequency of menstruation-related migraine attacks and missed workdays, days with reduced work productivity, and days when household duties or social activities were compromised has been demonstrated.

Significant social and professional consequences of menstruation-related migraine, and in particular, menstruation-predominant migraine have been previously reported. A significant percentage of employers reported that employees with migraines are less productive, and a portion reported that people affected by migraines had a higher absentee rate. It is also suggested that specific assessment of quality of life is needed in people with menstruation-related migraine, as using less comprehensive measures of life satisfaction causes part of the total estimated burden of chronic headache to be underestimated. Feelings of loss of productivity and frequent failure to meet life goals centered around career and job satisfaction, as well as problems with interpersonal relationships and leisure, were in part related to the burden of chronic migraine. Moreover, the personal environment and the need for the availability of support systems were perceived as important and may include work accommodations as part of an individually tailored therapeutic approach, job accommodations, and modification of the environment if people with menstruation-related migraine seek care, help, and relief from their often chronic disorder.

Migraines are complex and chronic headaches that usually affect only one side of the head and can last between 4 to 72 hours with a combination of symptoms, such as photophobia, nausea, and the presence of an aura prior to an attack. Despite the extensive list of symptoms, the primary symptom is throbbing pain that affects the quality of life. Emotional effects are related to menstruation-related migraines. Menstruation-related migraines affect the quality of life by causing emotional effects such as frustration, anguish, isolation, helplessness, and people often worry about the impact of migraines on family, career, and personal life. The throbbing pain, along with the multiple symptoms mentioned above, can cause people with migraines to be more debilitated than women with non-menstrual migraines. This is corroborated by a study where premonitory symptoms were more frequent in women with menstrual migraines than in women without menstrual migraines. In the same study, women reported aura symptoms, and similarly, in another study, subgroups of women complained of a lack of ability to work or perform social duties. Even though there is no recent article describing the social life of women living with menstrual migraines, it is extremely possible that due to the severe and frequent nature of the headaches, someone could feel more isolated from their friends and peers. Literature from other chronic pain conditions suggests that social life can be impacted by severe pain, with someone slowly withdrawing from society and also not participating in social activities. When the individual did not submit an answer to the closed question, there was a reduction in social activities. It is possible that the frequency and severity of attacks have also led to a degree of withdrawal from social circles. Thirty-one patients concurred that they would cancel various planned family, social, and work functions due to increased headaches on menstrual days.

Migraine episodes may result in challenges maintaining employment. The majority of the literature in the area of menstruation-related migraine and work and social impairment relates to lost productivity. A survey of employed women found that 97% reported absenteeism from migraine necessitating days off work and that 28% reported presenteeism with migraines. MH-associated presenteeism within the workplace leads to reduced effectiveness in workplace productivity, represented by presenteeism. In a study of migraines, just over half of working-age individuals with daily or near-daily episodic migraine worked fewer days due to migraine within the past year.

It is crucial that the understanding of the work and social implications of migraine expands from an economic perspective alone. Migraine leads to productivity losses and absenteeism. Although presenteeism was briefly mentioned, there is not much attention dedicated to this. To encourage a prevention-based workplace culture that promotes productivity and flourishing work environments, policymakers need to appreciate the challenges associated with presenteeism. It is vital to recognize that menstruation-related migraines can also have implications outside the workplace. Data showing that migraines reduce work productivity are certainly interesting, but what about the social activities and underlying routines that really contribute to our vast workforce? Mental load, such as housework, is also relevant to the educational sector, with findings showing that 24-29% of students reported that menstruation substantially reduced their learning capacity.

Menstruation-related migraines have multiple pathophysiological mechanisms; thus, the discovery of a novel target for treatment should provide additional options to identify individuals who respond to novel medication. The novel therapeutic targets and approaches on menstrual migraine focus attention on medication enhancing estradiol levels, reducing menstrual withdrawal response, recovering the desensitization of postsynaptic receptors, preventing presynaptic release of neuromediators, and antagonizing glutamine-induced central sensitization. The potential of personalized therapy has been demonstrated for certain groups of substances, including triptans and NSAIDs. This observation opens new therapeutic avenues based on the concept of individually tailored and targeted therapies. Still, a substantial amount of work is required to develop these findings.

This subsection shows that, in the coming years, we may expect a shift in the understanding and management of menstrual-related migraine (MR-M). Real-world evidence suggests that most women living with migraine self-identify which are MR-M almost via trial and error, for example, by trying to add triptans or nonsteroidal anti-inflammatory drugs outside of a suspected menstruation window. The advent of several mobile health applications empowering patients to track, for example, migraine episodes, steroid hormone levels, and incoming menses, based on their forecasting algorithms, points in a different direction. Specifically, the coupling of the natural history and presenting features of individual patients with that of historical cohort data offers the possibility to predict in advance when is the most likely day across the cycle a given person could develop a migraine attack and to intervene in a personalized, targeted, and proactive way. Remarkably, such proof-of-principle applications alerted the user several days before the expected visit of the attack, suggesting non-pharmacological lifestyle changes to implement, as well as pharmacological options.

CONCLUSION

The role of specialists for women with MR-M or refractory migraine is also clear. However, there is no cure for MR-M, and even with a clear suspected link, larger numbers of subjects were unable to have their condition improved. These individuals might not be accessing specialists early enough, in line with what was found for the time to this clinic, suggesting the need for a smoother clinic pathway. The effect of lifestyle changes was not under investigation, and this is an area of growing interest and potential. The Australian Federal Government has announced an initiative that will allow for access to digital mental health services. Digital health enables the integration of technology-based interventions into routine clinical practice and empowers people to manage their health. However, it also comes with challenges, including privacy of data and accessibility of technology support.

Future work and emerging technologies in this field include telehealth services. However, there was no evidence of these capabilities at the beginning of the survey. Emerging technologies that leverage wearable sensors and integrated algorithms to monitor real-time health changes may offer real-time feedback to prevent a migraine attack. Recommendations include increasing awareness and understanding of migraine headache approaches and severity, including available digital health solutions. Studies investigating digital health solutions for women with menstrual-related migraine are limited by sample sizes and heterogeneity of the interventions and outcome measures. The results of this review cannot be conclusively used to guide clinical practice, and the best method of applying digital health to the management of menstrual-related migraine is yet to be determined.

REFERENCES

Abreu-Sánchez, A., Parra-Fernández, M. L., Onieva-Zafra, M. D., & Fernández-Martínez, E. (2020). Perception of menstrual normality and abnormality in Spanish female nursing students. International Journal of Environmental Research and Public Health, 17(17), 6432.

Dahlgren, M. K., Smith, R. T., Kosereisoglu, D., Sagar, K. A., Lambros, A. M., El-Abboud, C., & Gruber, S. A. (2024). A survey-based, quasi-experimental study assessing a high-cannabidiol suppository for menstrual-related pain and discomfort. npj Women's Health, 2(1), 29.

Das, A. & Majhi, T. K. (). Problems Of Menstruation Among Adolescent Girls in Urban and Rural Community of Kamrup District, Assam. pdfs.semanticscholar.org.

Hirata, K., Sano, H., Kondo, H., Shibasaki, Y., & Koga, N. (2022). Impact of primary headache on daily activities: an observational study using online survey and medical claims data in Japan. researchsquare.com

Hirata, K., Sano, H., Kondo, H., Shibasaki, Y., & Koga, N. (2023). Clinical characteristics, medication use, and impact of primary headache on daily activities: an observational study using linked online survey and medical claims data …. BMC neurology.

Mavhunga, K. (2023). Prevalence of menstrual disorders and health-seeking behaviour of adolescents in Greater Giyani Municipality, Limpopo Province.

Sakai, F., Hirata, K., Igarashi, H., Takeshima, T., Nakayama, T., Sano, H., ... & Koga, N. (2022). A study to investigate the prevalence of headache disorders and migraine among people registered in a health insurance association in Japan. The Journal of Headache and Pain, 23(1), 70.

Sakai, F., Hirata, K., Igarashi, H., Takeshima, T., Nakayama, T., Sano, H., ... & Koga, N. (2022). A survey conducted using health claims records and questionnaires to investigate the prevalence of migraine in Japan.

Tomlinson, M. (2022). A study utilizing mixed methods to evaluate menstrual hygiene management and its impact on school attendance among schoolgirls in Edo State, Nigeria

AGBIOL 2024 CONFERENCE STUDENT ORGANIZING TEAM

#	NAME
1.	Emrah Akpınar
2.	Metin Burak Tatlıses
3.	Deniz Kızılkaya
4.	Mutlu Şen
5.	Yaren Akpınar
6.	Selinay Demir
7.	İlayda Küçük
8.	Neslihan Yılmaz
9.	Begüm Kurt
10.	Serhat Kayan
11.	Ece Zeren

OUR SPONSORS

PLATINUM SPONSOR

TRAGEN RD CO Ltd

www.tragen.gen.tr

We get support from TUBITAK